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# LIFE AND MICROGRAVITY SCIENCES

## SPACELAB MISSION:

### HUMAN RESEARCH PILOT STUDY

### *Six Month Report*

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Edited by

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## Preface

This report was designed to record the overall experience of the pilot study for the human experiments planned for the Life Sciences Microgravity Spacelab (LMS) mission. It is written for the personnel involved in the mission primarily, i.e. astronauts, scientists, and managers. Our effort was to assemble an informative and readable document including both general and technical aspects of the study.

The principle authors are the scientists for the mission who not only came to Ames Research Center to perform their experiments, but also managed to process their data and deliver preliminary results within a few months of completing the study. Our thanks to them for being so responsive and sensitive to the need for a six month report to collate the variety of experiments and preliminary results for the mission.

Drs. Joan Vernikos, Director Life Sciences Division, and Victor Schneider, LMS Program Scientist, initiated the pilot study at a time when most considered such an effort a year or so too late to be of benefit to the mission. The successful completion of the study with their support and with the invaluable assistance of Angela Jackman, LMS Life Sciences Program Manager, 10 mo before the planned launch, reflects the hard work of a large number of people (see acknowledgements). The driving spirit in the course of this work was largely due to general agreement with Drs. Vernikos and Schneider of the importance of this type of study for high quality reportable scientific research. The local support provided by Dr. Emily Holton, acting Chief of SLR Division, and Dr. Alan Hargens, Branch Chief, was essential to the timely performance of the study.

We have tried to incorporate the positive spirit in which the pilot study was done in this document without compromising the accuracy and value of the scientific data and achievements.



Karen R. Walker and Sara B. Arnaud, M.D.



# LIFE AND MICROGRAVITY SCIENCES SPACELAB MISSION: HUMAN RESEARCH PILOT STUDY

## *Six Month Report*

### Introduction

Pilot studies have been carried out for animal experiments on Cosmos 2044 and 2229, but this is the first pilot study conducted for a set of flight experiments using human subjects. The pilot study concept is to mimic the flight procedures following the planned mission sequence using flight-like hardware, when possible. The value of carrying out this ground-based performance of the flight investigation is that it provides the opportunity to test the full set of experiments in a combined sequence prior to the actual flight. In this way, procedures can be refined, timelines can be optimized and potential conflicts and incompatibilities discovered and resolved before launch. It also provides investigators with simulation data for comparison with flight to validate the suitability of the bed rest model in simulating microgravity on each physiological system under investigation.



*The LMS Bed Rest study was a collaborative effort involving the test subjects, investigative teams, facility support personnel, project leaders, and the LMS mission payload specialists.*

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### Contents

Introduction .....	1
Integrated Muscle Studies .....	6
E920 .....	9
E036 .....	12
E401 .....	16
E407 .....	20
E029 .....	24
E409 .....	26
Metabolism Studies .....	28
E030 .....	30
E971 .....	33
E074 .....	36
Performance Studies .....	39
E948 .....	40
E963 .....	43
E073 .....	47
Value of Bed Rest Pilot Study .....	48
Acknowledgements .....	52

Previous studies on Skylab, Spacelab (SLS-1 and SLS-2), and the Russian Cosmos and Mir missions have documented that weightlessness produces significant muscle weakness and wasting, particularly in the antigravity muscles. However, a comprehensive description of microgravity induced changes in human skeletal muscle structure and function and the contributing cellular mechanisms is not established.

The priority of the LMS mission investigation is the evaluation of the function and structure of skeletal muscle and the organ systems essential to muscular activity, i.e., the bone, lung, endocrine and central nervous systems. The ground-based pilot study used the 6° head-down tilt bed rest model to simulate space flight. Muscle structure and function were evaluated directly by a set of six "muscle" experiments. A second set of three experiments grouped as "metabolism" studies evaluated pulmonary function, energy metabolism and bone, and a third set of three experiments assessed the "performance" of the central nervous system before, during and after 17 d of bed rest. (The mission is planned for 16 d, with 2 additional days reserved for contingencies). The pilot study mimicked the experimental procedures as closely as possible and used the equipment available for ground-based research as well as some of the equipment for the mission.

The sequence of procedures for the pilot study are described using the time frame and terminology for the mission sequence (i.e., L-3 indicates the procedure was carried out during the "pre-flight" or control period, 3 d prior to the commencement of "launch" or bed rest; L+3 indicates the third day of bed rest, R+3 indicates the third "post-flight" or recovery day). Subjects were used as their own controls with data collection before, during, and after bed rest. To accommodate the testing schedule, the eight subjects were admitted in two groups of four, 2 d apart, and followed the same testing sequence.

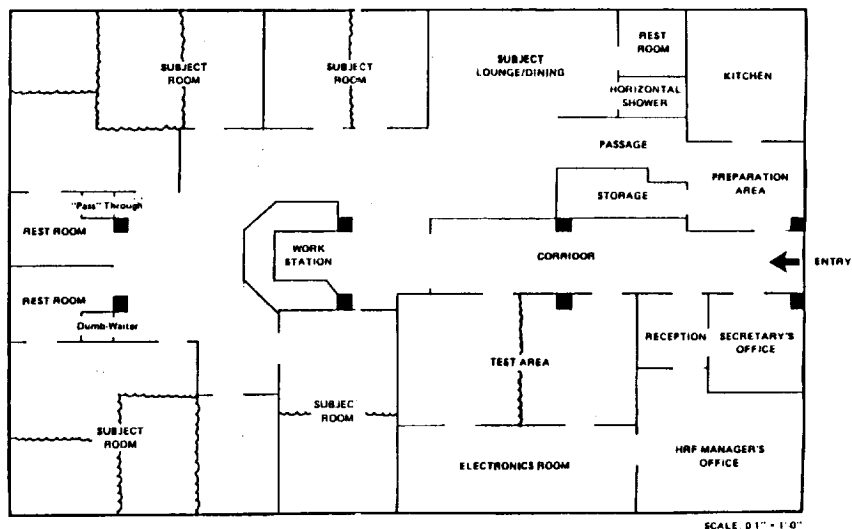
### Duration

The study took place between 19 June, 1995 and 28 August, 1995, according to the schedule at right:

Orientation Period	(Introduction, protocols, training): 19 - 30 June.	
Control Period	Group I: 1 - 25 July	Group II: 13 - 27 July
Bed Rest Period	Group I: 30 Jul-11 Aug	Group II: 27 Jul-12 Aug
Recovery Period	Group I: 12 - 26 Aug	Group II: 14 - 28 Aug

### Human Research Facility

This study was carried out in the Human Research Facility (HRF) at Ames Research Center, Moffett Field, California. Each subject was provided with reading materials (books, magazines, and newspapers), FM radio, and color television mounted on the ceiling. Individual selection of radio and TV stations was possible. Videocassette movies were transmitted to the individual TV sets from a centrally located recorder. Subject rooms had fluorescent lighting (on 0700 hr, off 2300 hr, 20-50 ft-candles) at the head of each bed, and indirect incandescent lights above.



The HRF consists of four rooms for housing bed rest subjects, a common dining and recreation area, three bathrooms, a central nursing station, test room, manager's office, kitchen, and small clean-up area.

### Control Period

[illegible]

## Bed Rest Period

[illegible]

## Recovery Period

		Aug '95														
Activity Name		Sat 12	Sun 13	Mon 14	Tues 15	Wed 16	Thurs 17	Fri 18	Sat 19	Sun 20	Mon 21	Tues 22	Wed 23	Thurs 24	Fri 25	Sat 26
Relationship to Launch Timeline		R+0	R+1	R+2	R+3	R+4	R+5	R+6	R+7	R+8	R+9	R+10	R+11	R+12	R+13	R+14
LeBlanc	029		●———●													
Egerton	036			●———●				●———●				●———●				
Fitts	920			●———●				●———●								
Tesch	409		●													●
dIPrampero	407			●———●				●———●				●———●				
Cerretelli	401		●													●
West	030	●		●———●				●———●								
Stein	971					●										
Cann	074	●	●	●———●				●———●				●				
Monk	948	10 hr	24 hr													
Schliffert	963	●		●		●		●								
Heschke	073	●								●						
			●———●		2 groups of 2 subjects each											
			●		4 subjects											
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

*Bed rest study schedule for four subjects in Group I. The remaining four subjects in Group II performed the same sequence of testing 2 d after Group I.*

## Personnel

The nursing staff provided round the clock medical care for 45 d with eight RNs and nine nurse's aids. The laboratory staff consisted of the clinical laboratory temporary supervisor and nine technicians who acquired, processed and stored the biological specimens for the scientists.

### *Daily Routine*

Vital signs were taken for each subject every morning upon awakening. Scheduled blood draws were carried out before the subject had risen. Subjects were then asked to empty their bladders and urine samples were collected. Following body weight measurements, breakfast was served. At particular times depending upon the protocols, subjects were required to eat breakfast at a given time. During the bed rest portion of the study, all testing, showering and excretory functions were carried out in the 6° head-down tilt position, standing up or sitting was not permitted. During the orientation, control and recovery periods, all subjects remained active and ambulatory.

### *Subject Selection, Preparation and Training*

Eight candidates averaging  $42.3 \pm 8$  years of age who were non-smokers, took no medications or drugs and passed a physical examination, that included a stress EKG test, qualified for admission to the study. All but one of the subjects (#457) had participated in previous bed rest studies. Training to use exercise equipment, pulmonary function tests, computer programs for

performance and record-keeping was carried out during the 2-wk Orientation period prior to admission to the unit. Informed consent for the experiments was obtained prior to the orientation following a detailed explanation of the procedures by investigators who were available to answer subjects' questions.

### Diets

Meals were prepared on site by a staff of four supervised by a research dietician. The nutrient composition of the diets considered the needs of the metabolic experiments, E074 and E971, as well as reported data from shuttle flights (Lane et al, Am J Clin Nut 60: 8015, 1994). A set of 12 daily menus was constructed to provide each subject 2500 Kilocalories, 86 g protein, 357 g carbohydrate, 84 g fat, 4200 mg (181 Meq) sodium, 3500 mg (100 Meq) potassium, 800 mg calcium and 1200 mg phosphorus. The menus were randomly distributed during the 45 d of the study. Snacks (cookies, fruit, granola bars) were available between meals and were passed twice a day during bed rest. Subjects kept daily records of all food consumed.

Nutrient	Control	Bed Rest	Recovery
Water, ml	1895±269	1640±366*	2036±437
Kilocalories	2779±296	2444±332*	2735±229
Protein, g	92±6	85±8*	89±6
Carbohydrate, g	429±54	353±52*	413±48
Fat, g	84±10	78±15	84±7
Sodium, mg	4194±428	3958±555*	4232±462
Potassium, mg	3597±275	3429±576	3477±283
Calcium, mg	839±120	732±152*	838±84
Phosphorus, mg	1244±120	1200±157*	1282±77

Water and all nutrients except fat and potassium were less during bed rest than control and recovery periods ( $P < 0.05$ ; paired  $t$ -test). Reduced water consumption did not appear to be due to limited access to water on muscle test days during bed rest.

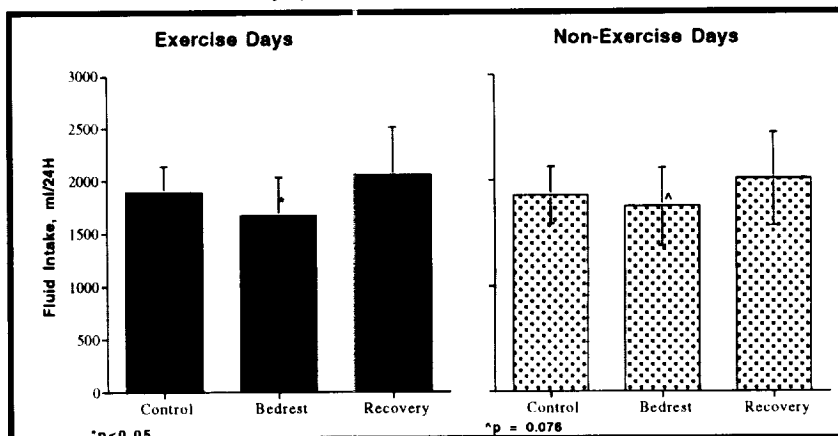
Subjects			
ID #	Age, yrs	Ht, cm	Wt, kg
439	45	185	99.5
435	41	178	77.3
420	51	188	88.4
374	49	170	68.1
457	25	178	66.5
249	40	188	75.5
405	46	183	90.7*
428	41	185	92.0**

\* Subject developed an acute febrile illness on the 9th bed rest day (L+9) with nausea and vomiting. He temporarily cancelled all testing sessions, but remained in head-down tilt bed rest. He was well enough to continue the procedures on the 1st day of the recovery period (R+0).

\*\* Subject developed an acute illness manifested by vertigo and loss of appetite on the 16th day of bed rest (L+16). His symptoms lasted for 7 d and limited his participation in scheduled experiments.

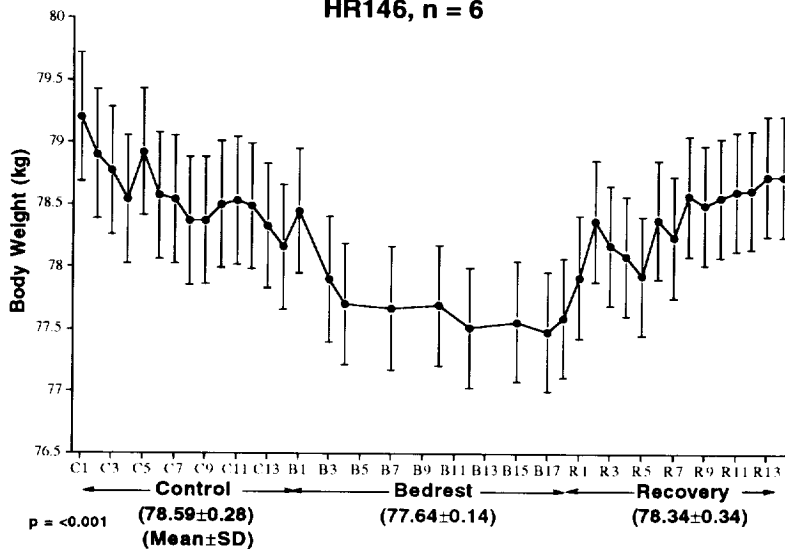
Average daily diets in six bed rest subjects unaffected by illnesses. Data are from an analysis of each subject's daily records by San Jose State University students of Dr. M. Navidi's class, "Nutrition in space". Values include snacks.

Fluid consumed on days with and without muscle tests was the same during each of the three time periods and showed similar differences during bed rest compared to control and recovery (paired  $t$ -test values are shown in the figure).

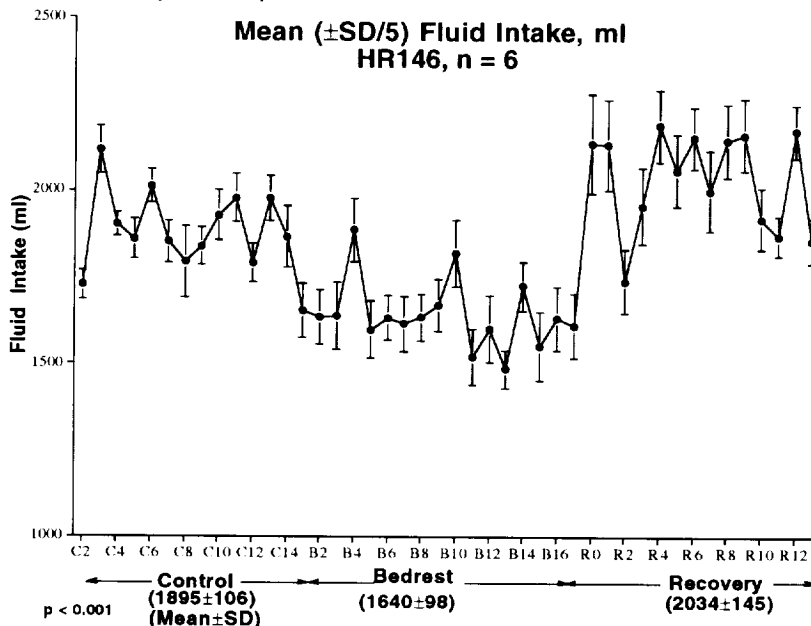




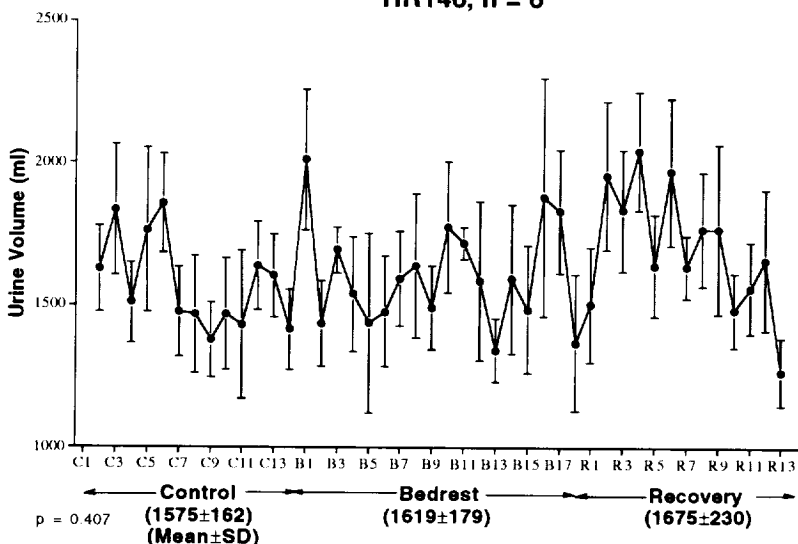
**Mean ( $\pm$ SE/10) Daily Body Weights, kg  
HR146, n = 6**



**Mean ( $\pm$ SD/5) Fluid Intake, ml  
HR146, n = 6**



**Mean ( $\pm$ SE) 24 Hour Urine Volume, ml  
HR146, n = 6**



## Body Weight

Weight loss averaging 1% of body weight was observed during the first 3 d of bed rest in six well subjects and was promptly regained during the first two recovery days. This pattern of weight loss during bed rest is typical and usually attributed to negative water balances during the first days of bed rest (Greenleaf et al., J Appl Phys 72:1887, 1992).

In this study, fluid intakes were depressed about 250 ml. Urine volumes were similar in all three periods except for an increase on the first day of bed rest ( $P=0.009$ ). The return of pre-bed rest body weight was prevented in one subject and delayed in the other because of the acute illnesses they suffered.

# Integrated Muscle Studies

## Overview

by Scott Trappe, Ph.D.

**S**keletal muscle can be considered a machine which transforms chemical energy into mechanical energy. This process takes place during muscle contraction which is normally triggered by voluntarily induced nerve stimulation.

The skeletal muscle project is composed of six experiments that were independently selected following peer review. Subsequently, at scientific working group meetings, the individual projects were modified in order to form an integrated project, and fit the necessary constraints of the space flight time line. The six experiments include three NASA (E029 Dr. LeBlanc, E036 Dr. Edgerton, and E920 Dr. Fitts), and three ESA (E401 Dr. Cerretelli, E407 Dr. di Prampero, and E409 Dr. Tesch) projects. Together these experiments investigate both the flexor and extensor muscle groups acting at the ankle, knee, wrist, and elbow. This design allows for a comparison of the effects of microgravity on muscle groups that normally bear different loads under physiologic conditions on earth. Although each project has its own focus and specific hypotheses, collectively they provide a detailed analysis of the relative importance of microgravity induced changes in contractile mechanisms, recruitment, motor perception, hormonal, and cellular factors mediating the reduced performance of the limb muscle groups studied.

The E029 and E409 experiments involve pre- and post-flight, but no in-flight analysis. Dr. LeBlanc (E029) employs MRI to directly assess the effects of microgravity on muscle cross-sectional area and volume. Dr. Tesch (E409) studies the functional properties of the knee extensor muscles. All of the other studies employ in-flight as well as pre- and post-flight measurements. In addition, these experiments (E036, E401, E407, and E920) are planned for the torque velocity dynamometer (TVD) which allows isometric, isotonic, and isokinetic contractions, with concentric and eccentric motions possible in the latter two modes. Experiments E036 and E920 study the right arm and leg, while E401 and E407 study the right arm and left leg.

Dr. Edgerton's experiment (E036) centers on the hypothesis that at least a portion of the decline in limb muscle function is due to an altered motor function. Specifically, it is hypothesized that the ability to achieve a target force or maintain a given limb position is compromised. Continuous recording of EMG also provide important information on the degree of motor recruitment and the relative importance of various agonists in maintaining a given torque. Dr. Fitts' experiments (E920) test the hypothesis that the primary factors reducing limb muscle function can be attributed to alterations at the cellular level. In particular, the decline in peak torque and power of the muscle can be attributed to a large extent

## Investigators MUSCLE

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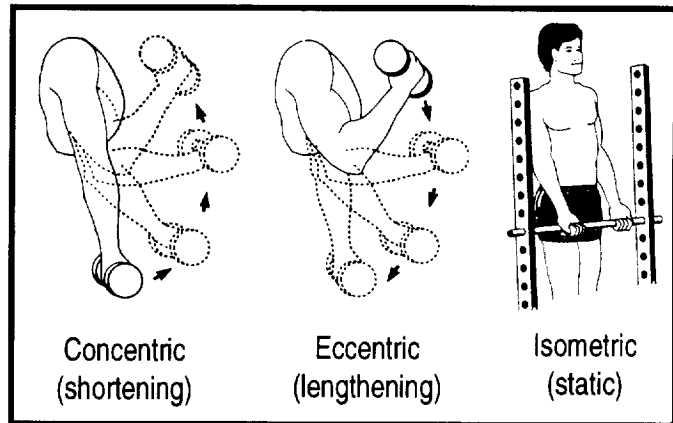
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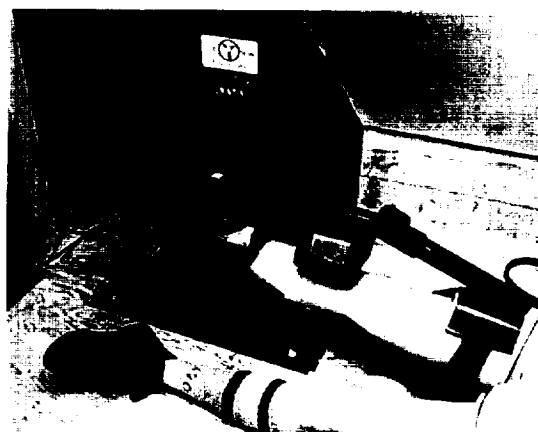
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to cell atrophy and to a reduced force per cross-sectional area caused by a selective loss in the contractile proteins. Additionally, it is hypothesized that microgravity results in a more rapid limb muscle fatigue due to both a percentage increase in the more fatigable fast fibers and to an inhibition in the ability to metabolize fats. Dr. di Prampero (E407) uses the TVD to conduct a series of experiments on both the elbow and the ankle. In both cases the specific aims are to determine the effects of weightlessness on: 1) the maximal voluntary isometric force; 2) the force-length relationship of the contractile component; 3) the force-velocity relationship of the contractile component; 4) the force-length relationship of the series elastic component; and 5) the motor unit recruitment pattern. The primary focus of Dr. Cerretelli's (E401) project is to study the muscle mechanics independent of voluntary control. Thus, the calf muscle group is activated by percutaneous electrical stimulation. This method allows the investigator to study both the individual twitch responses at different joint angles the relationship between force and frequency of stimulation and the fatigue characteristics of the calf muscle independent of central factors.



*The three main types of muscle contractions.*

A particularly important feature of the muscle research projects is that the individual experiments, although important in their own right, are further strengthened by their collective contributions. For example, it is hypothesized that microgravity leads to preferential atrophy of the slow-twitch muscle fibers, and to an increase in the percentage of fast-twitch muscle fibers. This hypothesis is directly tested by the cellular studies of Dr. Fitts, and by the limb dynamometer studies. A selective atrophy of slow skeletal muscle fibers coupled with an increased percentage of fast skeletal muscle fibers would be manifested in Dr. Cerretelli's experiments as a decrease in the time to peak tension and relaxation time, a right shift in the frequency-force relationship, and increased fatigability. In addition, differences in atrophy of the soleus and gastrocnemius can be detected by decreases in muscle volume (E029). Potential changes in EMG activity to torque ratios during measurement of the contractile properties and the fatigue test may also be unique to those individuals with relatively high or low proportions of slow fibers. Finally, the amount of atrophy that occurs in selected skeletal muscles (based on MRI, single cell measurements, and torque determinations) can be directly correlated with the level of muscle activation that occurs during routine movements in a normal day's tasks at 1-g and in-flight.



*Two dynamometers (LIDO (left) and Cybex (right)) were used for the LMS bed rest investigation. Experiments E036, E401, and E407 used the LIDO, while E920 used the Cybex unit. These units were located in different rooms within the facility and therefore simultaneous testing occurred in some instances.*

## Data Acquisition System

The system used for the acquisition of data from the LIDO Isokinetic dynamometer in E401 and E407 was DATAQ (DATAQ Instruments, Inc, Akron, OH). It was also used to acquire EMG data from the subjects (E407). The system was chosen because it was compatible with Windows and easy to use without sacrificing data acquisition power, programming, flexibility, and incremental performance.

## Muscle Testing Schedule

The integrated muscle experiment schedule is followed by brief reports of the individual projects as adapted to the bed rest pilot study. The pre-bed rest, bed rest, and post-bed rest time lines shown are judged optimal within the constraints of the time available for testing. The bed rest test sequence was established to allow for the time course changes in skeletal muscle function to be assessed.

Due to the rather large testing load, the testing was divided into a 2x2 matrix. During a scheduled testing time point, two subjects engaged in muscle measurements (E036, E920, E407 and E401), while the other two subjects completed the pulmonary function (E030) and  $\text{VO}_2\text{max}$  test (E920). The following day, the subjects switched and completed the opposite set of tests. The testing time points for the musculoskeletal experiments were as follows:

<b>Control Days</b>	2/3 (L-12/13) and 7/8 (L-7/8)
<b>Bed Rest Days</b>	2/3 (L+2/3), 8/9 (L+8/9), and 13/14 (L+13/14)
<b>Recovery Days</b>	2/3 (R+2/3), 6/7 (R+6/7), and 10/11 (R+10/11)

Muscle Protocol	Friday, July 14, 1995												Start Time	Finish Time
	7 AM	8 AM	9 AM	10 AM	11 AM	12 PM	1 PM	2 PM	3 PM	4 PM	5 PM	6 PM		
E036 (Edgerton) LIDO	[Timeline bar from 7:30 AM to 8:40 AM]												7:30 AM	8:40 AM
Motor control- calf	[Timeline bar from 8:40 AM to 9:50 AM]												8:40 AM	9:50 AM
E920 (Fitts) CYBEX	[Timeline bar from 9:00 AM to 9:35 AM]												9:00 AM	9:35 AM
Muscle strength - calf	[Timeline bar from 10:05 AM to 10:40 AM]												10:05 AM	10:40 AM
Reconfigure LIDO	[Timeline bar from 9:50 AM to 9:56 AM]												9:50 AM	9:56 AM
E407 (diPrampero) LIDO	[Timeline bar from 10:00 AM to 11:15 AM]												10:00 AM	11:15 AM
Force velocity -calf	[Timeline bar from 12:00 PM to 1:15 PM]												12:00 PM	1:15 PM
E401 (Cerretelli)	[Timeline bar from 11:16 AM to 12:00 PM]												11:16 AM	12:00 PM
Muscle stimulation	[Timeline bar from 1:15 PM to 2:00 PM]												1:15 PM	2:00 PM
Reconfigure LIDO	[Timeline bar from 2:00 PM to 2:05 PM]												2:00 PM	2:05 PM
E036 (Edgerton) LIDO	[Timeline bar from 2:05 PM to 3:00 PM]												2:05 PM	3:00 PM
Motor control - arm	[Timeline bar from 3:00 PM to 3:45 PM]												3:00 PM	3:45 PM
E407 (diPrampero) LIDO	[Timeline bar from 5:00 PM to 6:15 PM]												5:00 PM	6:15 PM
Force velocity - arm	[Timeline bar from 3:45 PM to 4:59 PM]												3:45 PM	4:59 PM
	[Timeline bar from 9:39 AM to 10:36 AM]												9:39 AM	10:36 AM
	[Timeline bar from 9:40 AM to 10:34 AM]												9:40 AM	10:34 AM
	[Timeline bar from 7 AM to 6 PM]													

This schedule shows the sequencing of muscle experiment tests for a given day.

The following abstracts were submitted to the 1996 American College of Sports Medicine Annual Meeting.

### Effect of 17 Day Bedrest on the Enzyme and Metabolite Profile of the Slow Type I Fiber

U.P. Grichko, R.H. Fitts, D.L. Costill, Dept. of Biology, Marquette Univ., Milwaukee, WI and Human Performance Lab, Ball State Univ., Muncie, IN

The purpose of this study was to determine if the bedrest model of weightlessness affects the enzyme and substrate profile in human skeletal muscle. Freeze-dried single fibers were prepared from human soleus biopsies before and after 17 d of bedrest. Each fiber was cut into three pieces for gel typing, metabolite and enzyme assay. Standard and digested fiber solutions were stored under oil in Teflon racks. Assays were conducted using fluorometric biochemical techniques based on pyridine nucleotide specific reactions followed, if necessary, by enzymatic cycling. The results for the slow type I fibers are shown in the table where  $\beta$ OAC =  $\beta$ -hydroxyacyl-CoA dehydrogenase, CAT=carnitine acetyl transferase, CS=citrate synthase, and GP=glycogen phosphorylase. The oxidative enzymes ( $\beta$ OAC & CS) showed a small but nonsignificant decline, while the FFA transport enzyme CAT was unaffected. Importantly, bedrest induced an ~1.5 fold increase in glycogen, and the enzymes involved in glucose and glycogen metabolism (hexokinase & GP) also significantly increased. These data are consistent with the hypothesis that both bedrest and weightlessness increase type I fiber dependence on carbohydrate metabolism.

	BOAC	CAT	CS	GP	Syn- thase	Hexo- kinase	LDH	PFK	ATP	Glyco- gen	Lac- tate	PCr
Pre	2.32	1.01	2.84	1.34	0.33	0.46	3.11	0.42	21.2	498.7	10.9	62.4
(49)	$\pm 0.24$	$\pm 0.13$	$\pm 0.29$	$\pm 0.19$	$\pm 0.46$	$\pm 0.06$	$\pm 0.29$	$\pm 0.06$	$\pm 1.3$	$\pm 38.1$	$\pm 1.0$	$\pm 3.7$
Post	2.16	1.06	2.38	2.08*	0.39	0.97*	3.64	0.40	17.6*	722.6*	21.0*	53.6
(55)	$\pm 0.27$	$\pm 0.11$	$\pm 0.17$	$\pm 0.20$	$\pm 0.04$	$\pm 0.16$	$\pm 0.36$	$\pm 0.04$	$\pm 1.2$	$\pm 40.5$	$\pm 2.2$	$\pm 3.9$

\* Pre vs post  $p \leq 0.05$ . Values are mean  $\pm$  SE.

Enzyme activity (mol/h kg dry wt) and substrate concentration (mmol/kg dry wt)

### Isotonic Contractile Properties of Soleus Muscle Fibers After 17 Days of Bedrest

J.G. Romatowski, J.J. Widrick, J. Sherwood, J.J. Bangart, D.L. Costill FACSM, R.H. Fitts FACSM, S.W. Trappe, T.A. Trappe, Dept. of Biology, Marquette Univ., and Human Performance Lab, Ball State Univ., Muncie, IN

Single permeabilized fibers were prepared from soleus muscle biopsies obtained from eight male subjects before and after 17 d of bedrest (BR). Fibers were mounted between a force transducer and servocontrolled position motor and subjected to 15-18 isotonic load clamps at maximal  $\text{Ca}^{2+}$  activation ( $15^\circ\text{C}$ ). The Hill equation was fit to data obtained from each individual fiber. Fiber myosin heavy chain (MHC) composition was subsequently determined by 5% SDS-PAGE. Force-velocity results from 205 type I pre and 127 type I post fibers revealed that BR induced a 14% reduction in fiber peak isometric force (from  $0.98 \pm 0.02$  to  $0.84 \pm 0.02$  mN,  $P < 0.05$ ), no change in the  $a/P_0$  ratio (pre  $0.050 \pm 0.003$ ; post  $0.044 \pm 0.003$ ;  $P > 0.05$ ), a 19% increase in fiber  $V_{\max}$  (the y-intercept of the force-velocity relationship, from  $0.53 \pm 0.02$  to  $0.63 \pm 0.03$  fiber length  $\cdot \text{s}^{-1}$ ,  $P < 0.05$ ), and a nonsignificant 7% decline in fiber peak power output (pre  $13.4 \pm 0.4$   $\mu\text{N} \cdot \text{fiber lengths} \cdot \text{s}^{-1}$ ; post  $12.5 \pm 0.5$   $\mu\text{N} \cdot \text{fiber lengths} \cdot \text{s}^{-1}$ ;  $P > 0.05$ ). This nonsignificant reduction in fiber power following human BR is substantially less than the 45-50% decline in type I fiber power observed after rodent hindlimb suspension. The increased  $V_{\max}$  of the human post-BR type I fibers appears to compensate for their loss in isometric force production and thereby serves to maintain fiber peak power at a normal weight bearing level.

## Peak Force and Maximal Shortening Velocity of Soleus Muscle Fibers after 17 Days of Bedrest

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**S**oleus biopsies were obtained from eight male subjects prior to and immediately after 17 d of 6° head-down bedrest (BR). Slack tests were performed on chemically permeabilized single fibers at maximal  $\text{Ca}^{2+}$ -activation (15° C). Each fiber was subsequently run on 5% SDS-PAGE to determine myosin heavy chain (MHC) composition. Only results from type I fibers are presented since this was the predominate MHC isoform expressed by both pre-BR (217 out of 227 total fibers) and post-BR (148 out of 171 total fibers) soleus fibers. BR induced a 5% reduction in soleus fiber diameter (from  $94 \pm 1$  to  $89 \pm 1$   $\mu\text{m}$ ,  $P < 0.05$ ), a 13% drop in fiber peak isometric force (from  $0.99 \pm 0.02$  to  $0.86 \pm 0.02$  mN,  $P < 0.05$ ), no change in fiber peak isometric tension (pre-BR =  $139 \pm 2$   $\text{kN} \cdot \text{m}^{-2}$ ; post-BR =  $138 \pm 3$   $\text{kN} \cdot \text{m}^{-2}$ ), and a 30% increase in fiber maximal unloaded shortening velocity ( $V_0$ , from  $0.86 \pm 0.02$  to  $1.12 \pm 0.04$  fiber length  $\cdot \text{s}^{-1}$ ,  $P < 0.05$ ). In conclusion, the decrease in the capacity of human soleus fibers to produce peak force following BR was attributable solely to a decrease in their size. This is in contrast to the rodent hindlimb suspension (HS) model which induces a loss in both fiber force and fiber tension. In addition, both BR and HS increase type I fiber  $V_0$ , although the relative increase following human BR is 40-75% less than that observed following 14-21 d of rodent HS.

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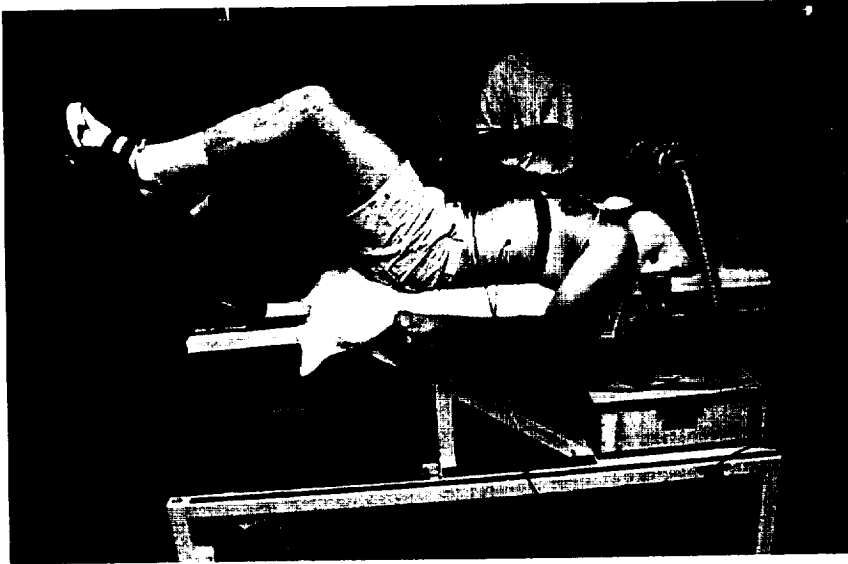
## Human Calf Muscle Function in Response to 17 Days of Bed Rest

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**A**s part of a project designed to mimic an upcoming spaceflight, 6° head-down tilt bed rest was conducted on eight health males for 17 d to assess the time course response of the in vivo contractile characteristics of the calf muscle group. The subjects' average age, height, weight, and supine cycling  $\text{VO}_{2\text{max}}$  were  $42.7 \pm 8.1$  y,  $182.3 \pm 6.5$  cm,  $82.2 \pm 12.1$  kg, and  $3.24 \pm 0.20$   $\text{L} \cdot \text{min}^{-1}$ , respectively. Calf muscle strength and fatigability characteristics were examined prior to bed rest, at the beginning (BR 2/3), middle (BR 8/9), and end (BR 13/14) of bed rest, and twice during recovery (R+2/3 and 7/8). Maximal isometric force was similar (-4 to -6%;  $P > 0.05$ ) during all time points at 80, 90, and 100° of ankle plantar flexion. Force production at six angular velocities (range = 0.52 to 5.24  $\text{rad} \cdot \text{s}^{-1}$ ) was also similar during bed rest. The decline in force production from a fatigue test consisting of 30 maximal contractions at 3.14  $\text{rad} \cdot \text{s}^{-1}$  was unchanged ( $72 \pm 3$  vs  $65 \pm 4\%$  on day 13/14), thus indicating the work capacity of the calf muscle was not compromised. Muscle biopsy specimens obtained from the soleus pre and on day 17 of bed rest indicated no change in muscle fiber composition, muscle fiber area, or capillary density. Citrate synthase activity decreased 20% ( $P < 0.05$ ) from  $122.1 \pm 7.8$  to  $97.1 \pm 5.1$   $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$ , while phosphorylase activity was unchanged. These data suggest that 17 d of bed rest did not alter whole muscle calf strength. In addition, these data contradict previous bed rest and leg suspension results, indicating that the time course testing used in this investigation may be sufficient in maintaining muscle function.

## Time Course of Cardiorespiratory Deconditioning with 17 Days of 6° Head Down Tilt Bedrest

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The purpose of this project was to assess the time course of exercise cardiorespiratory changes in response to 17 d of 6° head down tilt bedrest. Seven males (age,  $42.3 \pm 8.7$  y; height,  $181.4 \pm 6.5$  cm; weight,  $79.8 \pm 10.6$  kg) completed a continuous exercise test to volitional exhaustion on a supine cycling ergometer prior to bedrest (control), on days 2 or 3 of bedrest (BR 2/3, noted as L+2/3 elsewhere in this report), BR 8 or 9, and BR 13 or 14, as well as on days 3 or 4 of the recovery period (R+3/4), and R+7/8 to

determine the submaximal (150 W) and maximal cardiorespiratory responses to exercise. Submaximal oxygen consumption ( $\text{VO}_2$ ) and minute ventilation ( $V_E$ ) did not change from the control during bedrest or recovery. Heart rate (HR:  $\text{b} \cdot \text{min}^{-1}$ ) was significantly elevated ( $P < 0.05$ ) from the control ( $124 \pm 4$ ) during BR2 ( $134 \pm 4$ ), BR3 ( $138 \pm 4$ ), and R1 ( $135 \pm 2$ ), but decreased ( $P < 0.05$ ) from the control ( $3.24 \pm 0.20$ ) during BR1 ( $2.99 \pm 0.17$ ; -7.3%), BR2 ( $3.00 \pm 0.17$ ; -7.1%), BR3 ( $2.92 \pm 0.20$ ; -9.0%), R1 ( $3.02 \pm 0.20$ ; -6.6%), but was not different ( $P > 0.05$ ) than controls by R2 ( $3.13 \pm 0.19$ ; -3.3%).  $\text{HR}_{\text{max}}$  did not change from the controls during bedrest or recovery. Initial changes in  $\text{VO}_{2\text{max}}$  (BR1) were significantly correlated with urine balance during BR1 ( $r=0.91$ ,  $P < 0.05$ ). These results suggest that the time course for changes in cardiorespiratory responses to exercise are not linear and are related to the initial changes in body fluid volumes during 6° head down tilt bedrest.

## Relationship of Long-Term Electromyographic (EMG) Activity and Hormonal Function to Muscle Atrophy and Performance

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**E**036 was designed to determine the relationship between hormonal levels, global electromyographic (EMG) activity of a muscle and motor in healthy men before, during and after 17 d of bed rest. In the present paper, the authors looked more specifically at how muscle unloading during bed rest respectively influenced: 1) muscle activation, 2) motor control, and 3) growth hormone release.

### *Effect of Bed Rest on 24-Hour EMG Activity*

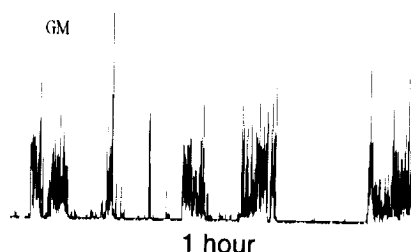
It was previously reported in animal studies that unloading with hindlimb suspension resulted in a short term reduction in the EMG activity of the ankle extensors, the soleus (SO) and gastrocnemius medialis (GM), and an increase in activity of their antagonist muscle, the tibialis anterior (TA). Moreover, the recruitment pattern of the SO and GM were altered during unloaded condition. In normal load bearing activity, the SO is recruited during both postural and low level activity whereas the GM muscle is activated mainly during activities requiring higher force-velocity outputs. During unloading, activity in GM is seen in the absence of soleus activity. The objectives of the 24-h EMG recording were: 1) to assess the level of activation of postural and non-postural muscles during normal activity and during prolonged bed rest; and, 2) to investigate the recruitment pattern of slow and fast ankle extensors. Considering the complexity of 24-h EMG signals analysis, this preliminary report does not attempt to draw conclusions from the data but rather provides a description of the analysis.

EMG activity of the SO, GM, TA, Biceps Brachii (BB), and Triceps Brachii (TB) muscles were recorded for 24-h periods pre, during and post 17 d of 6° incline bed rest. EMG signals were amplified (gain: 1000), and recorded on audiotapes with a TEAC HR-40 recorder. The data were acquired on a PC computer at a sampling rate of 1000 Hz. EMG signals were rectified, filtered and smoothed using a moving average and resulting in a matrix of integrated EMG amplitude per second (figure E036-a). EMG signals were further integrated for every hour to obtain a pattern of the behavior of muscle activation for 24 h (figure E036-b).

Comparison of hourly EMG activity was then made across sessions, within muscle and within subject to highlight effects of bed rest on the global amount of EMG activity as well



*The 24-h EMG test measured electrical activity of the muscles being monitored, and was conducted on the major muscle groups of the lower limb (tibialis anterior, gastrocnemius, and soleus) and upper arm (biceps brachii and triceps brachii) during the scheduled muscle testing days.*



*Figure E036-a.*



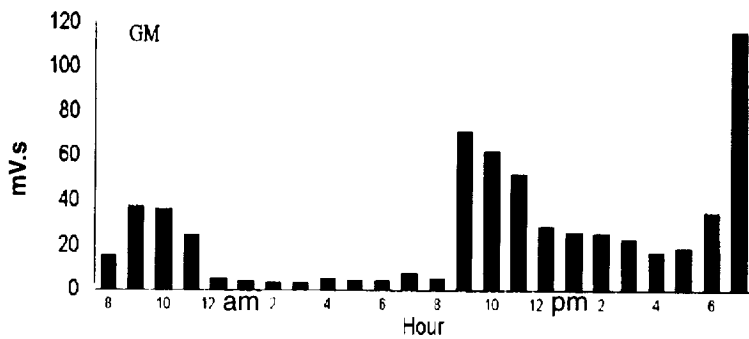


Figure E036-b.

as on the circadian pattern of this activity. Figure E036-c depicts for one subject the effects of bed rest on integrated EMG activity of the GM muscle. For this particular subject, the circadian rhythm does not seem to be affected by prolonged bed rest.

Finally, an estimate of global muscle activation was computed by integrating 24-h of EMG activity. The data were further filtered for noise, a filtering process that may alter the preliminary results.

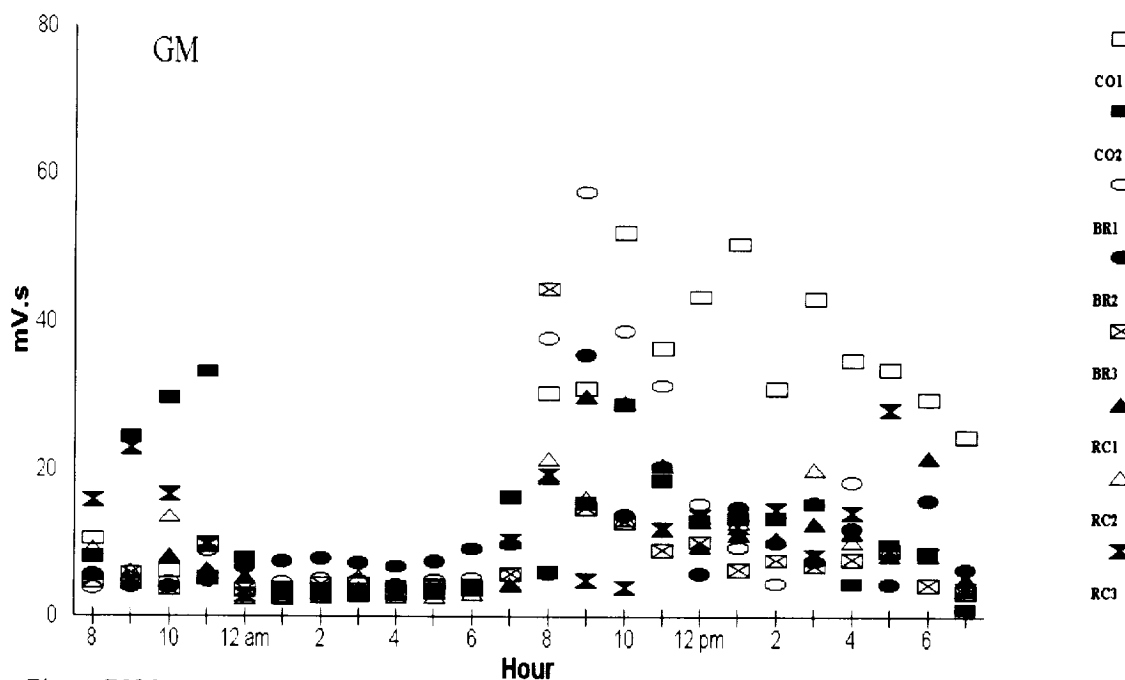


Figure E036-c.



During both the recovery and control testing periods, and for the bed rest period, subjects were tested in a supine position (6° incline with the head lower).

### ***Bed Rest Has No Effect on Perceived Muscle Output During Isometric Muscle Contraction***

One important determinant of 'typical' effort levels during many tasks is the force required to overcome gravity (g). Therefore, removal of gravity can be viewed as a marked perturbation to neural circuitry involved in perception of effort which evolved in a 1-g environment. Indeed, cosmonauts and astronauts have reported that the sense of effort is considerably altered during space flight and for a variable period following return to 1-g environment. As well, measurements of perceived effort during dry

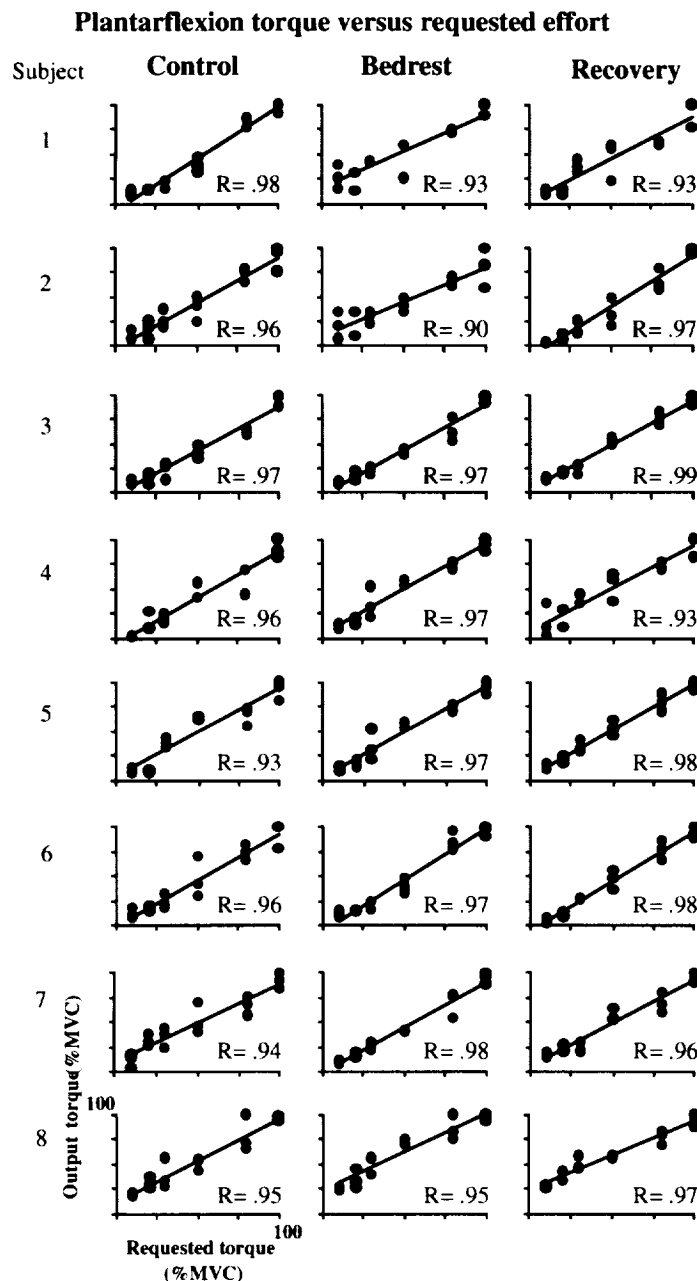


Figure E036-d.

relationships for the eight subjects during control, bed rest, and recovery periods. No significant change in the slope or y-intercept, nor in the correlation coefficient, of this relationship was found in a comparison of bed rest and control/recovery testing. It was concluded that perception of muscle output was not affected by bed rest in this study group. A somewhat surprising finding in this study was the remarkable acuity, in all subjects, of muscle output perception across the entire muscle output range. These findings emphasize the important question of whether or not the high level of muscle output discrimination would be lost or altered in microgravity. Presently, the authors are analyzing data from additional experiments, carried out in parallel during the above study, examining effects of bed rest on perception of joint position and on muscle force perception during non-isometric muscle contractions.

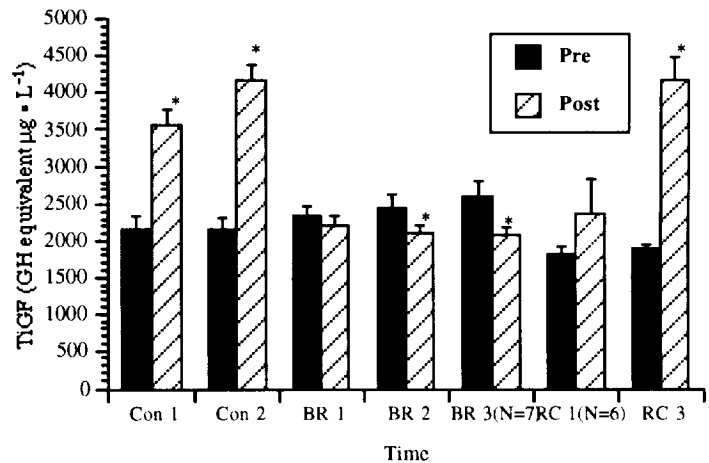
immersion experiments have suggested adaptation in nervous mechanisms giving rise to sense of effort. Quantitative analyses of the time course of these changes are lacking.

Bed rest provides a model of reduced muscle activity and has been used to simulate the altered muscle activity found in a microgravity environment. Physiological changes due to prolonged bed rest are also of interest to medical practitioners who wish to shorten the recovery time of patients under their care. Clinical observations of changes in muscle during prolonged bed rest range from reports of slight or moderate muscle atrophy to severe atrophy and contracture. No studies have been reported on the effect, if any, of prolonged bed rest on the perception of muscle force or effort during voluntary muscle contraction. The present study was undertaken to measure the effect of bed rest on perception of muscle output.

The experiment was divided into a lower limb portion, examining torque and EMG measurements from ankle dorsiflexors and plantar flexor muscles, and an upper limb portion examining torque and EMG measurements from elbow flexor and extensor muscles. Muscle contractions were isometric for all measurements reported in the present study. In the four functional muscle groups studied (plantar flexors, dorsiflexors, elbow flexors and extensors), no significant effect of bed rest on the perception of muscle output was found. Figure E036-d displays the requested effort versus muscle (plantar flexor) torque rela-

### Changes in Hormone Response to Muscle Activity During Bed Rest

This study investigated hormonal responses in eight men to a muscle fatigue test before [L-12 (Con1) and L-7 (Con2) days], during [L+ 2/3 (BR1), L+8/9 (BR2), and L+13/14 (BR3)] and following [R+2/3 (Rec1) and R+10/11 (Rec3) days] 17 d of bed rest. The fatigue test involved a series of unilateral isometric plantar flexions and included four maximal voluntary contractions (MVC), 48 contractions at 30% MVC, and 12 contractions at 80% MVC all performed at a 4:1 s work:rest interval. Additional motor control testing preceded the fatigue test. Blood was collected for hormonal analysis prior to motor control tests and immediately following the fatigue test. Growth hormone (GH) measured by radioimmunoassay was unaffected by the fatigue test during all testing periods. However, a hypophysectomized rat GH bioassay of tibial cartilage growth indicated a significant increase ( $P<0.05$ ) of a tibial growth factor (TiGF;  $\mu\text{g} \cdot \text{L}^{-1}$ ) following the fatigue test (Con1  $2146 \pm 192$  to  $3565 \pm 197$ ; Con2  $2162 \pm 159$  to  $4161 \pm 204$  (figure E036-e). This TiGF response was absent at BR1 and significantly decreased ( $P<0.05$ ) by BR2 ( $2433 \pm 185$  to  $2105 \pm 106$ ) and BR3 ( $2594 \pm 211$  to  $2085 \pm 109$ ). By Rec3, the TiGF response had returned ( $1881 \pm 75$  to  $4160 \pm 315$ ). Testosterone and thyroid hormones (T3 and T4) were unchanged during all testing periods. In conclusion, the release of TiGF in response to a fatigue test was inhibited during bed rest, but had returned by 10-11 days of recovery.



\* significantly different from pre (paired t-test;  $p<0.05$ )

Figure E036-e.

## Effects of Bed Rest Deconditioning on Human Calf Muscle Contractile Properties

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Paolo Barattini, M.D., Ph.D.

**B**ed rest is a commonly adopted model to simulate the conditions of microgravity. A potential problem with this and other models, such as limb suspension, is that the muscles are still subjected to the pull of gravity even though they are not bearing any weight. Nonetheless, evidence exists that bed rest as well as limb suspension result in disuse muscle atrophy that is quantitatively comparable to microgravity conditions of space flight (Musacchia et al., J Appl Phys 69: 2248, 1990; Riley et al., J Appl Phys 73, Suppl 33S, 1992). From a qualitative point of view, however, reservations exist as to whether limb suspension and bed rest induce a muscle atrophy comparable to that induced by space flight (Berg et al., J Appl Phys 70, 1882, 1991). Contrary to the finding of preferential slow-twitch fiber atrophy of rat muscle after space flight, the results of simulated microgravity in man seem to provoke a similar gross muscle atrophy but a non specific fiber atrophy since the decrease in type I and type II fibers CSA was similar after 4-6 wk of limb suspension (Hikida et al., Aviat Space Environ Med 60: 664, 1989; Hather et al., J Appl Phys 72: 1493, 1992). Despite the shortcomings of the models simulating microgravity conditions, bed rest still represents one of the most valid methods to mimic the response of humans to space flight. The purpose of the present study was therefore to investigate the effects of bed rest induced muscle atrophy on skeletal contractile properties in humans.

### *Test Schedule*

After becoming familiar with the equipment and procedure, subjects were tested on control days 13 (L-13) and 8 (L-8) before the start of the bed rest, on days 2 (L+2), 8 (L+8) and 13 (L+13) during the bed rest, and on days 2 (R+2), 6 (R+6) and 10 (R+10) of the recovery period.

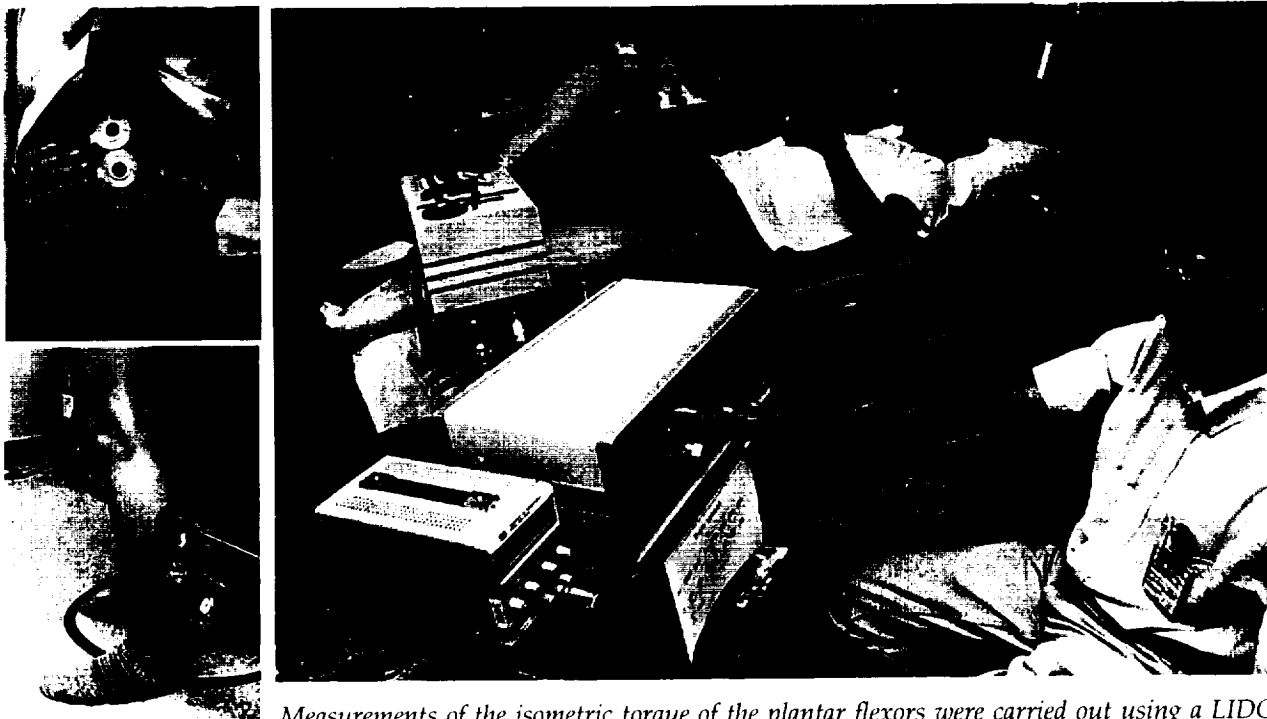
### *Experimental Procedure*

The functional properties of the triceps surae (TS) were investigated by recording the torque developed during isometric plantar flexions, during both voluntary and electrically evoked contractions. The triceps surae was stimulated percutaneously using two large disposable electrodes, the cathode over the gastrocnemius and the anode over the soleus. A Percutaneous Muscle Stimulator (PEMS) delivered a square-wave monophasic 50  $\mu$ sec pulse that was subject selectable from 100 mA to 800 mA, by 50 mA steps through two electrodes attached to the subject's leg. Electrical stimulation allowed the study of the contractile properties of the plantar flexors independently of the volition of the subject providing an objective measure of the functional status of the muscle. The protocol was as follows:

### *Investigated Parameters*

**Supramaximal Current Intensity (SMCI).** To determine the threshold current required to activate all motor units the muscle group was stimulated with increasing current intensity from 100 mA up to a 800 mA (in 50 mA steps) until no further increase in torque was observed - this level was identified as the SMCI.

**Angle-Force Relationship (AFR).** In order to assess the relationship between joint angle and force of the plantar flexors, the twitch force in response to single pulses at SMCI level were recorded at foot plate angles of 0, 5, 15, 20 ° of dorsiflexion (DF), and 5, 15, 25, 30° of plantar flexion (PF).



*Measurements of the isometric torque of the plantar flexors were carried out using a LIDO dynamometer (Loredan, California) by setting the angular velocity to zero. All measurements were performed with the knee positioned at 90° of flexion (above right). A Percutaneous Electrical Muscle Stimulator (PEMS) was interfaced to surface electrodes as illustrated (bottom left). Ground electrodes were also placed on the hip area (upper left).*

**Twitch Characteristics.** The time to peak tension (TPT), half-relaxation time and rate of rise of tension ( $dT/dt$ ) were measured for twitches generated at 15° of dorsiflexion. This angle was chosen because it corresponds to the optimum angle for peak twitch force of the ankle flexors.

**Maximum Voluntary Contraction (MVC).** Three isometric MVCs, each lasting 4 s with 20- s intervals in between, were then recorded at 20° DF. During each voluntary contraction, the degree of muscle activation was assessed using the twitch superimposition technique by which two single stimuli at SMCI, interspaced by 1.5 s, were delivered. Maximality of the voluntary contraction was defined by the disappearance of the twitch.

**Frequency-Force Relation (FFR).** The frequency-force relation (FFR) of the muscle was investigated by stimulating the TS at 60% SMCI with three single twitches, 5 s apart, followed by consecutive 1 s trains at 10, 20, 30, and 50 Hz. This lower current intensity, 60% of the SMCI, was used to minimize the subject discomfort at the higher frequencies.

**Fatiguability.** The fatiguability of the TS was evaluated during 2-min intermittent stimulation at 60% SMCI with trains of pulses at 20 Hz delivered every second for 350 ms. A fatigue index (FI) was calculated as the ratio between the torque of the last contraction (120th) over that of the first.

### ***Statistics***

The data are expressed as means  $\pm$ SD. Significance of the differences was analyzed with one way ANOVA. The Fisher's protected test was used to locate differences between means with significance set for  $P < 0.05$ .

## Results and Discussion

**SMCI.** The current required to activate all motor units essentially remained constant throughout the study and averaged  $635 \pm 100$  mA.

**AFR.** Significant changes ( $P < 0.001$ - $0.05$ ) in the AFR were observed during the bed rest and recovery periods (figure at right). The torques developed at 20, 15 and 5° of dorsiflexion and at 5° of plantar flexion after 2, 8 and 13 d of bed rest were significantly lower than those recorded during the baseline data collection at L-8. The maximum decrease in twitch force was 23.4% at 15° of dorsiflexion, on L+8. Torque values were still lower on R+6, by 23.4%, and interestingly had not recovered to baseline values on R+10, thus indicating that 10 d of recovery were not sufficient for full recovery of muscle strength. A particular noteworthy observation was that the loss of torque occurred mainly for dorsiflexion angles and not, except for 5 PF, for plantar flexion angles. Since the AFR of the plantar flexors results from the individual length-force (L-F) relations of the gastrocnemius and the soleus, greater atrophy of one of these muscles could have changed the shape of the AFR.

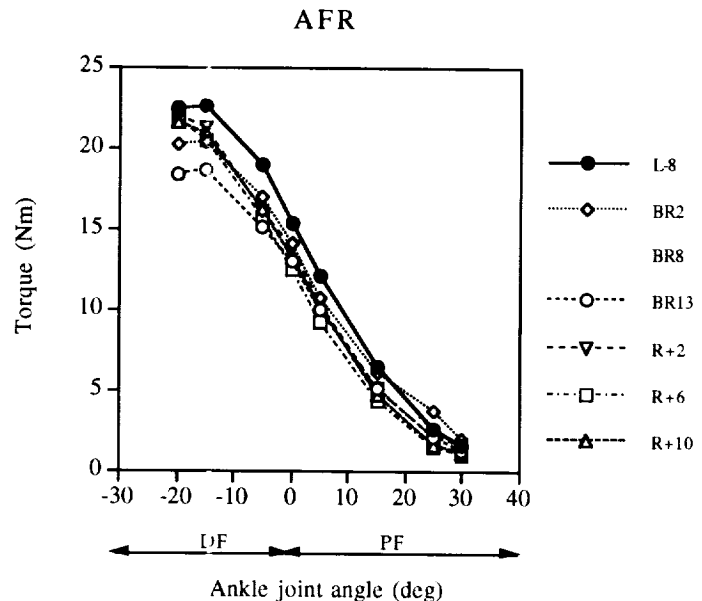


Figure E401-a.

**Twitch Characteristics.** Twitch torque ( $T_{tw}$ ) was decreased by 23% ( $P < 0.001$ ) and 18% ( $P < 0.001$ ) after 8 and 13 d of bedrest; on R+10 torque values were still lower by 8% ( $P < 0.05$ ) than those recorded on L-8. The decrease in  $T_{tw}$  was likely due to muscle atrophy. The PF muscle plus bone cross-sectional area (m+b CSA) measured by anthropometry at R+2 was found to be decreased by 8%. A slight decrease in TPT was observed on L+2 and L+8 compared to L-8. TPT values on R+2 and R+6 were significantly longer than those of L+2 and L+8 but still comparable to that of L-8. The rate of tension raise,  $dT/dt$ , significantly decreased during the bed rest period by as much as 20% ( $P < 0.01$ ) on L+13. This decrease in  $dT/dt$  was no longer significant once values were normalized for maximum  $T_{tw}$ , thus indicating that this effect was related to muscle atrophy. During recovery these differences were reduced but were still lower by 9.5% ( $P < 0.05$ ) on R+10.

There was a progressive reduction in half-relaxation time during recovery on R+2, R+6, and R+10 whereby  $1/2RT$  was reduced by 7%, 9%, and 8%, respectively. The decrease in  $1/2RT$  seems rather difficult to reconcile with the reduction in  $dT/dt$  but is likely attributable to different mechanisms acting independently.

**Maximum Voluntary Contraction.** Although no significant changes in MVC were observed throughout the study (figure E401-b), it was found that the ability of the subjects to voluntarily activate all motor units of the PF was low at L-8 and progressively improved with time. In fact, 3 out of 8 subjects showed force potentiation by the effect of the interpolated twitch at L-8, thus showing incomplete motor units activation. The number of subjects showing an effect of the twitch decreased to 1 out of 8 at L+8 and R+2. No effect of the interpolated twitch was found on R+10. Therefore, the fact that MVC did not change during the bed rest seems due to a poor ability to activate all motor units in some of the subjects at the beginning of the study. This hypothesis seems substantiated by the fact that the tetanic force at 50 Hz decreased by 17.4% between L-8 and R+2. A reduction in MVC would indeed have been expected from the 8% decrease in m+b CSA of the IF observed on R+2.

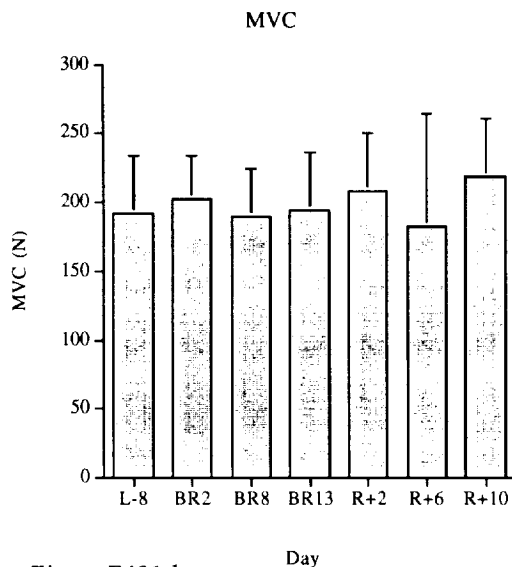


Figure E401-b.

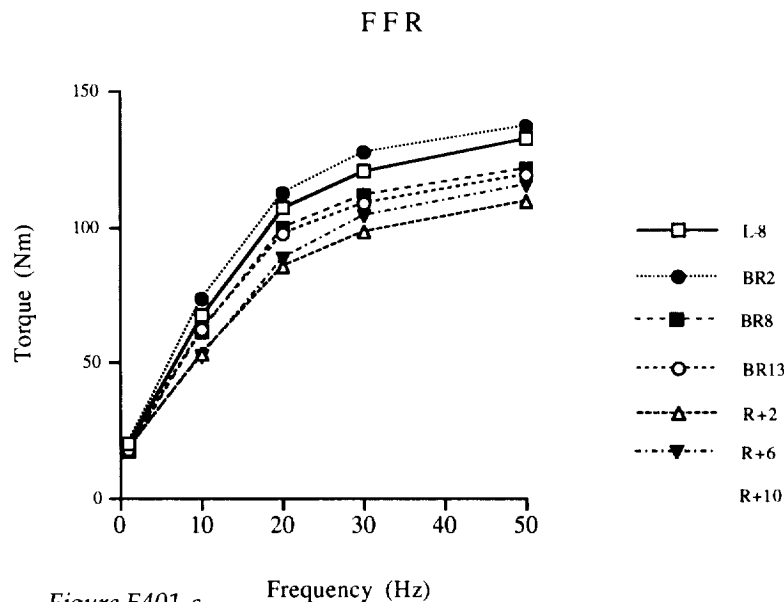


Figure E401-c.

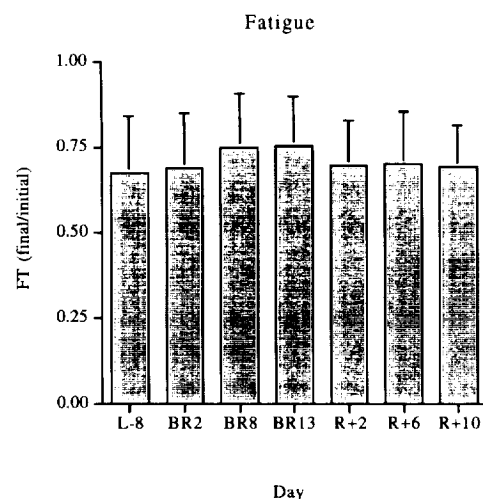


Figure E401-d.

**Frequency-Force Relation.** A progressive decrease in the force at all stimulation frequencies was found during the bed rest and also during the recovery period (figure E401-c). The largest decrease in force was found on R+2 at which the tetanic force at 50Hz ( $F_{50\text{Hz}}$ ) had decreased by 17.4%. It was noteworthy that on R+10 the tetanic force had not fully recovered to baseline values, since  $F_{50\text{Hz}}$  was still 6% lower than the value on L-8. The loss of force at 1, 10, 20, 30 and 50 Hz was proportionally the same, in fact once the force at each frequency was normalized for the force at 50Hz, the shape of the force-frequency curves obtained on all sessions perfectly overlapped. From these observations it was concluded that the loss of force was likely due to a non-specific muscle atrophy. Had one population of fibers undergone atrophy to a greater extent than another a greater loss of force at specific frequencies should have been observed.

**Fatiguability.** A decrease in fatiguability of 11-12% ( $P < 0.05$ ) was observed on L+8 and L+13 (figure E401-d). Fatiguability then returned to baseline values from R+2 onwards. The decrease in muscle fatigue observed towards the end of the bed rest period and the quick reversal to baseline values on recovery seem to suggest that metabolic causes rather than structural alterations are responsible for this phenomenon. Mechanical factors seem unlikely candidates since on L+8 and L+13, the half-relaxation time, which if increased would reduce fatiguability, was not significantly different from that of L-8.

## Conclusions

This study has shown that 17 d of bed rest resulted in a 23.4% loss of plantar flexors twitch tension. The decrease in twitch tension was joint angle-specific and modified the angle-torque relation of this muscle group. A 17.4% loss of tetanic torque was observed but no change in the frequency-force relationship was found suggesting a non-specific fiber atrophy. The decrease in tetanic torque was correlated with a 8% decrease in muscle plus bone cross-sectional area. Twitch force and tetanic force were still below baseline values after 10 d of recovery. A decrease in fatiguability during bed rest and recovery was observed.

## Effects of Microgravity on the Biomechanical and Bioenergetic Characteristics of Human Skeletal Muscle

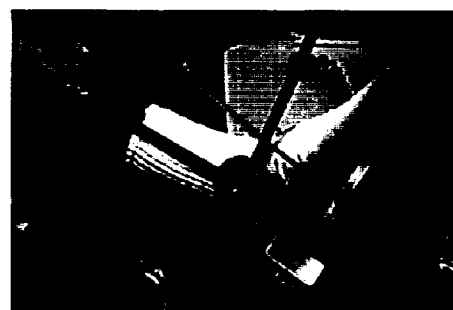
Stefania Milesi, M.D., Carlo Capelli, M.D., Edgar Stussi, Ph.D., Jachen Denoth, Ph.D., and Pietro di Prampero, M.D.

**H**ead-down bed rest has been customarily utilized to mimic on Earth the effects of exposure to microgravity and it can effectively simulate the hypokinesia and hypodynamia occurring during space flight (Greenleaf and Kozlowski, *Med Sci Sports Exerc* 10:94, 1982; Sandler and Vernikos, *Inactivity: physiological effects*. Academic press, New York, 1986; Dudley et al., *Aviat Space Environ Med* 60:659, 1989). Therefore, in the present study, the effect of short term head-down bed rest on the biomechanical characteristics of the human skeletal muscles was investigated. Torque developed by the extensors and flexors of the ankle and the elbow joints, along with their electromyographic activity, was measured during isometric and isokinetic contractions.

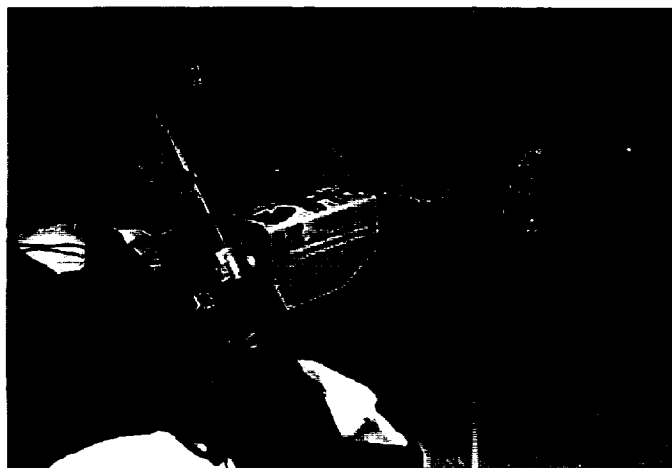
### Methods

**Torque.** Isometric and isokinetic muscle torque of the flexors and extensors of the left ankle and of the right elbow was determined by a LIDO dynamometer.

**EMG.** Myoelectric surface signals were recorded from the lateral belly of the gastrocnemius, from the tibialis anterior and on the bellies of the biceps and triceps. The preamplified electrodes (Electromyographic System, Model 544, Therapeutic Unlimited, Iowa) were positioned according to the standardization proposed by Zipp (Zipp, *Eur J Appl Phys* 50:41-54, 1982). The following three separate protocols were used to assess the four muscle groups:



*For the isometric protocols, subjects laid in the supine position on the bench of the LIDO and the angles of the knee and ankle joints were set at 90° (above). The position of the foot on the plate defined the reference point (0°) from which all the angles of the joint were measured during the experiments (left).*



**Protocol IM - ankle joint.** The subjects performed a maximal voluntary contraction (MVC) of 5-s duration with the tibialis anterior (TA) immediately followed by one with the triceps surae (TS). After 2 min of rest, the series of two MVCs was repeated. The joint ankle was moved to the new position and, after 2 min of rest, the subjects performed a new set of four MVCs. The isometric torque was determined at 25, 15 and 5° of plantar flexion (PF) and at 5 and 15° of dorsiflexion (DF). The sequence of the joint angles was randomized before each experiment.

**Protocol E/C - ankle joint.** In the concentric-eccentric isokinetic mode, the range of motion of the lever arm was preset between angles of 30° PF and 15° DF. Starting from full plantar flexion, the subject pulled maximally with the TA while the lever arm of the LIDO was moving at the preset angular speed of 60°/s (1 rad/s). Once the full DF was attained, the lever arm moved actively backward to full PF. The subjects were asked to keep full activation of the TA, therefore in this phase the TA was forcibly stretched. The exercise always began with a concentric contraction followed immediately by an eccentric one. This cycle was repeated from 4 to 6 times for a total duration of about 4-



6 s. After 3 min of pause, the protocol was performed with the TS. The subject rested again for 3 min then resumed exercising with the TA at the faster angular speed of  $230^\circ/\text{s}$  ( $4.02 \text{ rad/s}$ ). The experiments ended with the series at the same fast speed ( $230^\circ/\text{s}$ ) performed with the TS.

**Protocol C/C - ankle joint.** The same range of motion and angular speeds as in EC were tested in the isokinetic concentric-concentric mode. The subject moved the footplate, pulling with the TA and pushing with the TS, resulting in a concentric muscle contraction in both directions of movement. The trials at the two speeds consisted of 4 to 6 repetitions, timed 3 min apart.

After modifying the joint angles, the same protocols were applied to the elbow.

## Results

This preliminary report summarizes the results obtained from testing the ankle joint during the isometric protocol (IM) in the two control sessions and in the third bed rest session in all subjects, and during the isokinetic protocols (C/C and E/C) in 4 of 8 subjects.

Tibialis anterior		Triceps surae	
Position (deg)	avg Torque (Nm)	control	bed rest
25 PF	$32.6 \pm 1.5$	$32.8 \pm 1.8$	$40.7 \pm 7.2$
15 PF	$35.2 \pm 2.3$	$35.8 \pm 1.9$	$60.5 \pm 8.0$
5 PF	$37.5 \pm 2.9$	$36.7 \pm 2.0$	$81.9 \pm 8.3$
5 DF	$35.8 \pm 2.7$	$33.6 \pm 2.3$	$98.4 \pm 8.7$
15 DF	$31.8 \pm 2.0$	$29.1 \pm 2.0$	$111.8 \pm 14.9$

Tibialis anterior		Triceps surae	
Position (deg)	$\{dT/dt\}_{\max}$ (Nm·s <sup>-1</sup> )	control	bed rest
25 PF	$365.4 \pm 138.1$	$260.0 \pm 106.2$	$584.9 \pm 221.1$
15 PF	$424.2 \pm 160.3$	$345.7 \pm 141.1$	$755.2 \pm 285.4$
5 PF	$567.5 \pm 214.5$	$395.7 \pm 161.5$	$874.9 \pm 330.7$
5 DF	$492.3 \pm 186.1$	$326.6 \pm 146.0$	$978.7 \pm 369.9$
15 DF	$353.7 \pm 133.7$	$313.1 \pm 127.8$	$1022.9 \pm 386.6$

Table E407-a. Average values of  $T \pm SE$  of the two muscular groups tested in the isometric protocol.

**Isometric Protocol.** The average isometric Torque (T), measured during a 2-s long plateau phase following the fast T increment after the onset of the contraction is plotted in figure E407-a. The average values of  $T \pm SE$  of the two muscular groups are also reported in table E407-a. Eight subjects were tested in the control sessions and seven after 13 d of bed rest. A non-parametric Wilcoxon statistical test performed on the paired data showed no significant differences between the two conditions.

In figure E407-b, the maximal rates of increment of  $T \{dT/dt\}_{\max}$  at the onset of the contractions in the TA and TS are shown. These values are reported in table E407-a. Even though it was not possible to detect any significant difference between the average values at the various angles,  $dT/dt$  was systematically increased in the TA and decreased in the TS after bed rest.

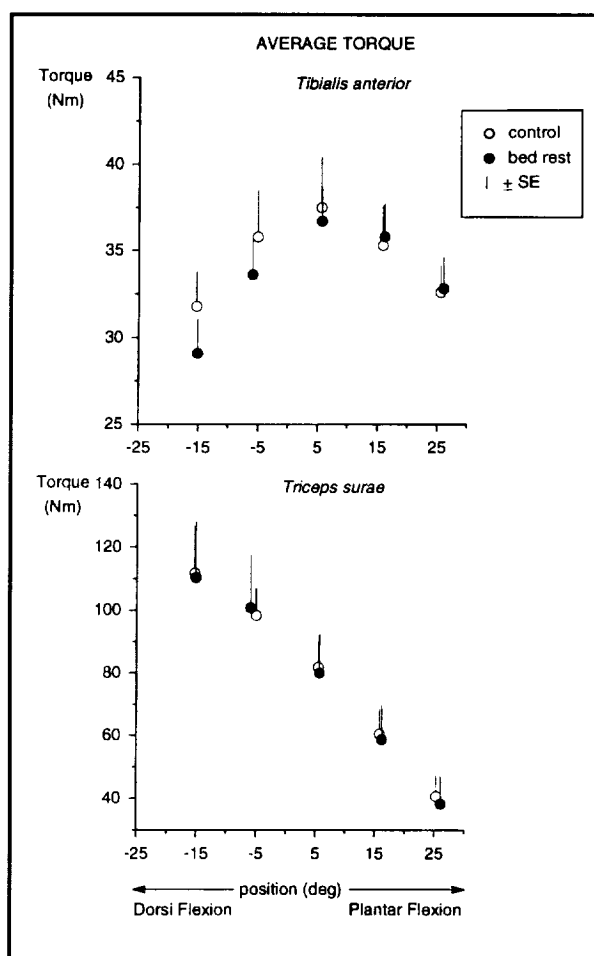


Figure E407-a The average values of the isometric torque of TA and TS as a function of the angular position in degrees.

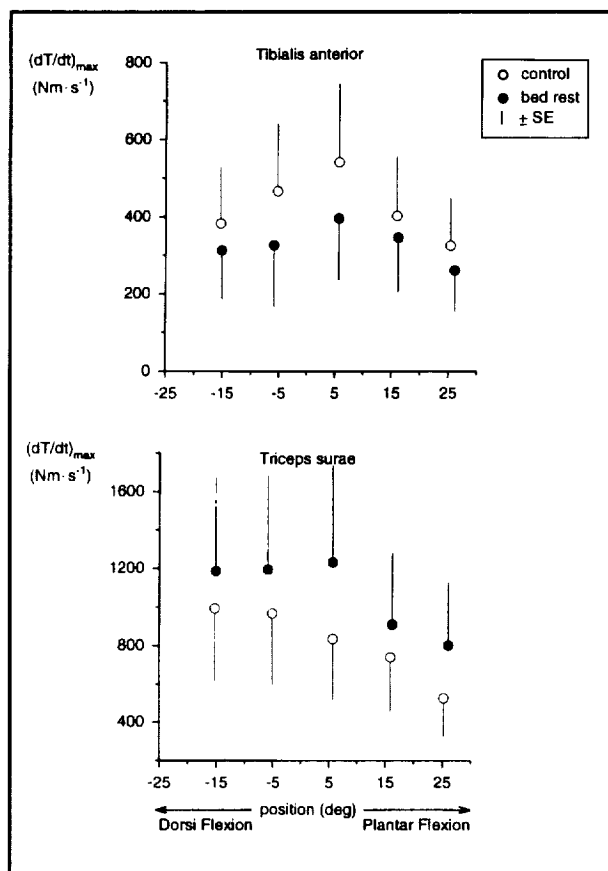


Figure E407-b. Maximal rate of increment of torque  $\{dT/dt\}_{max}$  at the onset of the contractions in the TA (upper panel) and in the TS (lower panel) as a function of the angular position.

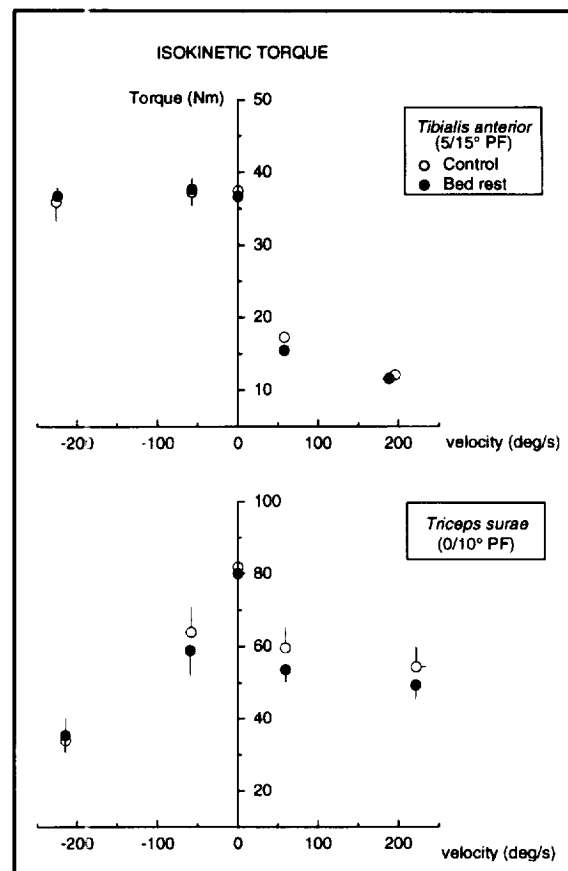


Figure E407-c. Average values of isokinetic torque of TA and TS, along with the corresponding isometric  $T$ , are plotted as a function of the corresponding angular speeds.

**Isokinetic Protocol.** The Torque, measured at the fixed ankle angle of  $10^\circ$  PF (range  $5$ - $15^\circ$  PF) for the TA and of  $5^\circ$  PF (range  $0$ - $10^\circ$  PF) for the TS was the average of the values obtained in three complete concentric-eccentric and concentric-concentric cycles. In figure E407-c it is plotted as a function of the corresponding angular speeds (positive values refer to concentric and negative values to eccentric muscular exercise).

### Comments

	Tibialis anterior			
	control		bed rest	
CON	196.1 $\pm$ 7.1	12.1 $\pm$ 0.6	188.4 $\pm$ 8.3	11.6 $\pm$ 0.8
CON	57.5 $\pm$ 0.3	17.3 $\pm$ 0.7	57.6 $\pm$ 0.4	15.5 $\pm$ 0.6
ISOM	0	37.5 $\pm$ 2.9	0	36.7 $\pm$ 2.0
ECC	57.5 $\pm$ 0.2	37.3 $\pm$ 1.8	57.3 $\pm$ 0.4	37.7 $\pm$ 1.5
ECC	226.7 $\pm$ 2.7	36.0 $\pm$ 2.6	224.7 $\pm$ 3.3	36.8 $\pm$ 1.2
	Triceps surae			
	control		bed rest	
CON	222.1 $\pm$ 12.0	54.4 $\pm$ 5.5	221.6 $\pm$ 2.3	49.4 $\pm$ 3.7
CON	58.9 $\pm$ 0.3	59.6 $\pm$ 5.7	59.5 $\pm$ 0.2	53.6 $\pm$ 3.4
ISOM	0	81.9 $\pm$ 8.3	0	80 $\pm$ 12.5
ECC	58.5 $\pm$ 0.2	64.1 $\pm$ 6.9	59.2 $\pm$ 0.1	58.9 $\pm$ 6.9
ECC	214.5 $\pm$ 1.6	34.0 $\pm$ 6.2	214.8 $\pm$ 0.4	35.4 $\pm$ 4.7

Table E407-b. Average  $T$  and speed values of the four subjects tested in the isokinetic protocol.

The data presented here were obtained only on the ankle joint and refer to the control sessions and to the last bed rest session. As such, they offer only a partial view of the effects of bed rest on the characteristics of the tested muscular groups. In essence, the paucity of the data can not indicate whether training or habituation effects occurred, thus reducing the amplitude of those induced by bed rest inactivity. This will hopefully be understood after the analysis of the entire data set. Moreover, the data regarding electromyographic activity of the TA and of the TS are being analyzed and were not presented here.

In spite of these facts, a general comment can be made. First, the 13 d head-down tilt period did not seem to impair the isometric T capability expressed during maximal voluntary contraction of the subjects, neither to alter the relationship between angular position and isometric T, both in the TA and in the TS. Second, the rate of increment of T at the onset of the isometric contraction decreased in TA and increased in TS after bed rest. These findings could be tentatively explained by the effect due to bed rest on the population of the muscle fibers in the TS. In fact, the disuse of antigravity muscles could have been responsible for shifting a substantial amount of type I fibers towards the characteristics of type II cells, which are endowed by a faster speed of contraction.

The opposite behavior found in the TA might be explained by an impairment of the recruitment pattern of its motor units. This possibility must be evaluated.

The relationship between isokinetic T and angular speed, both in eccentric and concentric contractions, does not appear to be altered after 13 d of bed rest in 4 of 8 subjects. The shape of the T velocity relationship in the TA is comparable to the one obtained by other investigators using monarticular isokinetic ergometers on different joints (Edgerton and Roy, *Adv in Space Biol and Med* 4:33-67, 1994). This is not the case with the TS, in which the T exerted during eccentric contractions is lower at the highest speed than at the lowest. This could be due to technical problems occurring when the eccentric/concentric protocol was performed with the TS. The software controlling the LIDO actuator did not always counteract the high T produced by the stretched muscles during the eccentric phase at the highest speed. This inconvenience often forced the subject to stop the exercise which could only be resumed at a lower level of activation. This hypothesis will be tested by analyzing the corresponding EMG traces.

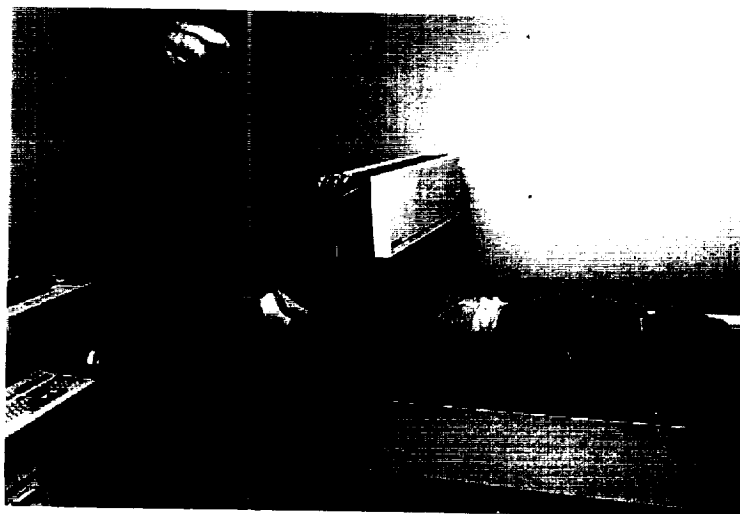
## Magnetic Resonance Imaging After Exposure to Microgravity (Bed Rest)

Adrian LeBlanc, Ph.D., Harlan Evans, Ph.D., Chen Lin, Ph.D., Linda Shackelford, M.D., and Thomas Hedrick, M.D.

This investigation tested the hypothesis that muscle cross-sectional area and volume are reduced during bed rest. Magnetic Resonance Imaging (MRI) was used to measure muscle mass, intervertebral disc space, bone marrow, and fluid distribution changes. This testing was only conducted pre- and post- bed rest.

### Dexa Results

All eight male subjects received a whole body scan using the Lunar scanner during the week prior to bed rest, on the last day of bed rest and again 24 to 48 h after reambulation. The data were analyzed for differences between these three time points and for three regions (total body, legs, total body minus the legs) using repeated measures ANOVA. In addition, the ratio of legs divided by total body minus legs was calculated and tested for each individual and time point. In instances where the ANOVA was significant, the data were further tested using the Tukey-Kramer Multiple Comparisons test. A comparison was considered significant at  $P < 0.05$ . The analyses included four types of measurements; bone mineral density (BMD), bone mineral content (BMC), fat, and lean body mass (LBM).



Dual Energy X-Ray Absorptiometry, or DEXA, scans for the bed rest study were made with the instrument at Ames Research Center, the Lunar DPX-L.

There were no significant changes in BMD, BMC, or fat. There were significant changes in the LBM as indicated in the table. We interpret these results to indicate that the fluid loss from bed rest came primarily from the legs, amounting to about 800 cc, and that the net % change in LBM was about equal from the legs and the remainder of the body.

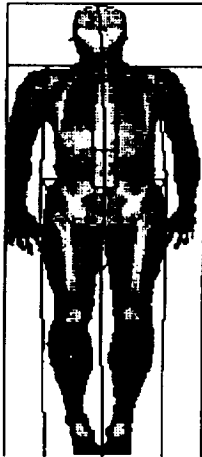
Lean Body Mass (LBM), g				
Schedule	L-5,6 d	L+16 d	R+1,2 d	
	Pre-Bed Rest	Bed Rest	Post-Bedrest	[overall Δ]
Total Body Minus Legs	39360	38678	38496	
Δ		-682	-182	-864
% Δ		-1.7	-0.4	-2.2
Legs	19231	17891	18712	
Δ		-1339	820	-519
% Δ		-7.0	4.7	-2.6

The legs lost 7% LBM on average during bed rest; approximately 5% was regained during the 24 - 48 h after reambulation with a remaining 2.6% loss when compared to pre-bed rest control average. The total body minus legs showed a significant unrecovered loss of about 2.2%. The ratio of leg LBM divided by whole body minus leg decreased during bed rest and returned to pre-bed rest values during the reambulation period.

**TOTAL BODY RESULTS**  
Ames Research Center

PATIENT ID: 249LMS  
NAME: 249LMS, NASA\_LMS

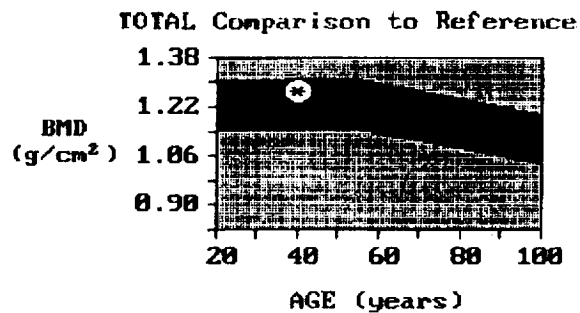
SCAN: 1.3z 07/22/95  
ANALYSIS: 1.3y 07/26/95



**LUNAR®**

IMAGE NOT FOR DIAGNOSIS

ID: 249LMS, NASA\_LMS SCAN DATE: 07/22/95



TOTAL BMD (g/cm <sup>2</sup> ) <sup>1</sup>	1.271 ± 0.01
TOTAL % Young Adult <sup>2</sup>	104 ± 3
TOTAL % Age Matched <sup>3</sup>	104 ± 3

Sample DEXA report.

### *MRI Results*

The vertebral length was measured between first and fifth lumbar vertebrae (L1 and L5). The total increase during bed rest was about 3.5 mm at the end of 2 wk bed rest (153.6 to 157.1 mm,  $P < 0.001$ ), greater than after overnight bed rest (2.7 mm), but less than long duration bed rest (4.4 mm). The magnitude of change appeared to increase from L1 to L5. After reambulation, vertebral column length was not different than baseline.

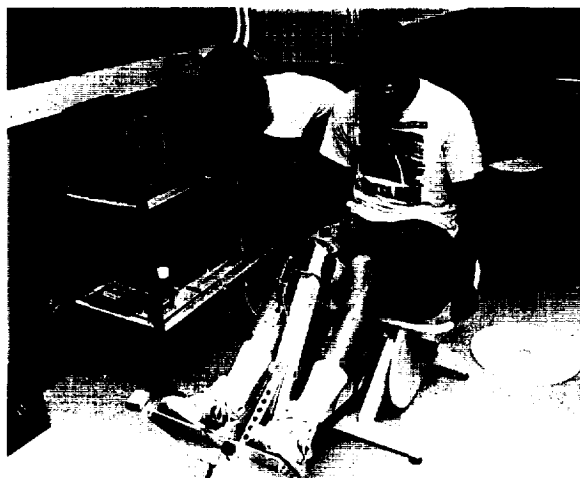
## Effects of 16 Days Bed Rest on Quadricep Muscle Function and Mass

Per Tesch, Ph.D. and Hans Berg, Ph.D.

The quadricep muscle undergoes marked atrophy in response to simulated space flight. Similarly, force production during maximal voluntary contractile activity is compromised and this effect appears to be of even greater magnitude. Hence, muscle atrophy cannot fully account for the impairment in muscle function shown in response to simulated space flight. This would suggest that additional factors, e.g., decreased neural drive and/or loss of contractile elements reduce the ability to generate force. This study was conducted to shed further light on the potential mechanisms that govern a decrease in muscle function in response to simulated space flight.

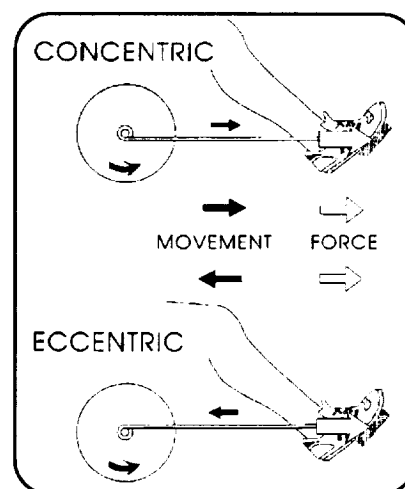
### Methods and Procedures

Force and power were measured during leg press exercise using a gravity-independent ergometer designed for use in space. Following an orientation session aimed at getting the subjects acquainted with the ergometer and the protocol to be used, two baseline sessions were performed prior to bed rest. Post measurements were carried out on R+1 and R+12 (1 and 12 d, respectively, after bed rest). Isometric (maximal voluntary contraction; MVC at 90° knee angle) and concentric and eccentric force using four different loads



The cycle ergometer used the inertial of one or more fly-wheel(s) to produce resistance during concentric and eccentric muscle actions.

were measured using strain-gauge technique. Also, EMG was recorded during a 60-s sustained isometric action at 30% MVC (pre-bed rest value). Muscle cross-sectional area (CSA) of m. quadriceps femoris and m. gluteus maximus was assessed by magnetic resonance imaging (see E029).



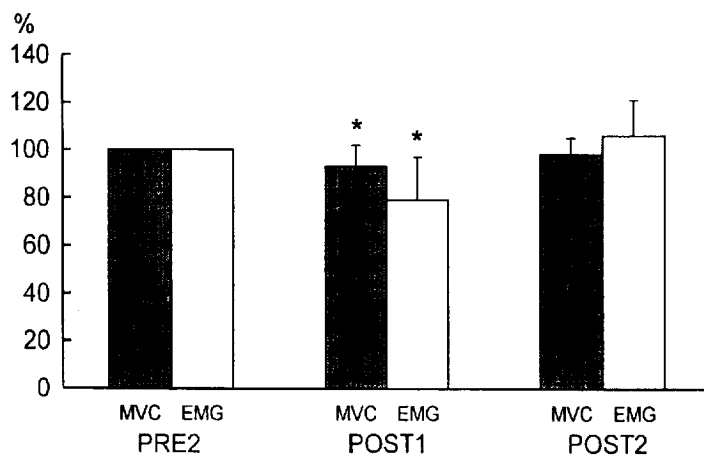
Force exerted during concentric muscle action (pushing movement; upper panel) increases the flywheel rotation and mechanical work is stored as kinetic energy ( $E_{kin} = 1/2 \cdot J \cdot \omega^2$ ; where  $J$  = flywheel rotational inertia and  $\omega$  = angular velocity of flywheel). An eccentric muscle action (braking movement) is then performed (lower panel) against the inertial force while aiming at retarding the flywheel rotation.



Electromyographic (EMG) activity, using bipolar surface electrodes, was recorded along with force from the three superficial heads of the quadricep muscle.

### Results

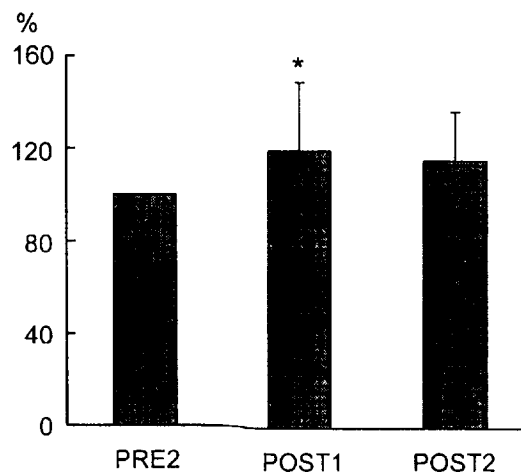
MVC decreased 8% ( $P < 0.05$ ) and maximal EMG was reduced by 21% ( $P < 0.05$ ) after bed rest. Following 12 d ambulation both MVC and EMG were normalized. EMG at 30% MVC increased 20% ( $P < 0.05$ ) after bed rest. It was normalized after 12 d ambulation. Force, power and EMG, measured during concentric and eccentric actions, remain to be analyzed. Changes in muscle CSA are pending results from E029.



Maximal isometric force (maximal voluntary contraction; MVC; shaded bars) and EMG (electromyographic; open bars) activity before (PRE2) and on R+1 (POST1) and R+12 (POST2) after bed rest. Values (mean  $\pm$  SEM) are normalized to PRE2. Star denotes a value which is significantly different ( $P < 0.05$ ) from PRE2.

EMG activity during a sustained isometric action at 30% MVC before (PRE2) and on R+1 (POST1) and R+12 (POST2) after bed rest. Values (mean  $\pm$  SEM) are normalized to PRE2. Star denotes a value which is significantly different ( $P < 0.05$ ) from PRE2.

EMG AT 30% MVC



## Conclusion

Bed rest (17 d) produced a significant decrease in isometric force of the quadricep muscle. This effect is accompanied by a decrease in neural drive, as reflected in EMG activity. Although these are important changes, the impairment in skeletal muscle function appears somewhat smaller than what could be predicted from previous studies using different models of unloading. Whether this effect is due to the fact that subjects performed multiple sessions of progressive supine cycle ergometer exercise during bed rest can only be speculated.

# Metabolism Studies

## Overview

The sources of energy for muscle contraction and work are carbohydrates, proteins and fat in food which are oxidized to carbon dioxide and water. Energy generated as work, heat, or storage for future use is commonly measured indirectly by quantifying the end-products of protein metabolism (nitrogen) and of biological oxidations ( $\text{CO}_2$  and water) or the amount of oxygen consumed. Since  $\text{O}_2$  is not stored, its consumption keeps pace with immediate needs and, assuming normal gas exchange in the lung, is an indication of the energy liberated (approximately 4.82 kcal/L  $\text{O}_2$  consumed at rest). The expectation is that muscle atrophy, induced by bed rest (and space flight) will reduce the capacity to perform work and will be revealed by reduced oxygen consumption during a period of muscular exertion. The factors affecting regional blood flows and pulmonary function associated with changes in posture during bed rest will necessarily have major effects on muscle contraction and/or work capacity.

Pulmonary function at 1-g is strongly affected by body position. Functional differences in the lung apex and base while upright diminish in the supine position. The cephalad shift of fluids is thought to be involved in a decrease in vital capacity, either through engorged pulmonary vessels or from a redistribution of fluids to the interstitial compartment in the lung. These effects of body posture can be revealed by the stress from exercise. In this study, experiment E920 tests oxygen consumption during exercise and E030, compares parameters of pulmonary function in the upright and head-down tilt positions.

Loss of muscle strength and lean body mass are reflected by negative nitrogen balance. While these deficits may also be reflected by decreases in body weight during bed rest, the fairly rapid loss and return of body weight observed in almost all bed rest study subjects has been attributed to losses in total body water (Greenleaf, NASA TM#103987, 1993). Negative nitrogen balances and decreases in lean body mass may not be associated with decreases in body weight because of increases in body fat, data that will be available to us through body composition measurements from E029. A second possible explanation for the increased breakdown of protein during bed rest (and space flight) are increases in stress hormones, part of the protocol of E948. A third cause of protein wasting could be nutritional, i.e., an energy deficit from either reduced intake or reduced expenditure. Analysis of dietary records have revealed a spontaneous modest decrease in intake which theoretically is an adjustment to reduced energy expenditure during bed rest. The technique that uses doubly labeled stable isotopes of hydrogen and oxygen to quantify energy expenditure in E971 will resolve some of these issues.

Reduced energy expenditure is likely to be accompanied by reduced body temperature, as reported in previous bed rest studies (Winget et al., J Appl Phys 33:639, 1972). The circadian pattern of changes in body temperature during bed rest is quantified in this study by E948, a study with both endocrine and neurosciences objectives (see Performance Section).

## Investigators

### METABOLISM

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**Dentistry of New Jersey**  
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**MD. Schluter**

**E074 • C.E. Cann, Ph.D.**  
**UCSF**  
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**C.D. Arnaud, M.D.**



Calcium and bone metabolism are intimately connected to muscle metabolism and sensitive to changes in body posture and activity. Losses in whole body calcium may not be measureable in as short a time as 2 wk and are localized to weight-bearing bone. The early responses in calcium metabolism to bed rest are monitored by the stable isotope studies of E074. This experiment will also obtain evidence for the effects of body position on bone turnover through assays of products of the osteoblast (bone forming cell) circulating in blood, and of collagen degradation excreted in the urine. Previous experience in the markers of bone metabolism reveal measureable increases in a sensitive circulating marker of bone metabolism, osteocalcin, and in urinary deoxypyridinoline in men exposed to bed rest with no exercise for only 1 wk (Leukens et al., J Bone and Mineral Res 8:1433, 1993).

## The Effect of -6° Head-Down Tilt on Cardiopulmonary Function

Janelle M. Fine, Ann R. Elliott, Ph.D., G. Kim Prisk, Ph.D., David L. Weber, Marc E. Mackey, and John B. West, Ph.D.

This study determined the effect of -6° head-down tilt (HDT) on various aspects of cardiopulmonary function. Cardiac output ( $Q_c$ ), stroke volume (SV), heart rate (HR), pulmonary tissue volume ( $V_t$ ), and residual volume (RV) were measured. Seven male subjects were studied while standing and supine twice during the control and bed rest periods and three times in the first week of recovery.

A specially designed pulmonary function test system with a bag-in-box configuration was used. Flow, gas concentrations, ECG, and arterial oxygen saturation were all measured with instruments calibrated before, during, and after each subject test session.



The reported parameters were measured by having the subjects rebreathe a gas mixture of 0.6%  $C_2H_2$ -10% Ar-21%  $O_2$ -bal  $N_2$ . The bag volume was specific to each subject (between 5.5 and 6.0 L), and the subjects rebreathed for 15 s at approximately 30 breaths/min after directing the breathing path into the test bag at residual volume. The results were analyzed using the method of

Sackner et al. (Am Rev of Resp Disease 3: 157, 1975). Results are presented as the parameters of interest normalized for each subject to his average pre-tilt standing value, and significance was accepted at  $P < 0.05$ .

	Pre Standing	Pre Supine	Tilt	Post Standing	PostSupine
<b><math>Q_c</math> (l/min)</b>	6.37±0.22	7.25±0.20*	7.51±0.15*	5.44±0.18*	6.89±0.16+
<b>SV (ml)</b>	72±2	98±3*	104±3*	58±2*	89±3
<b>HR (beats/min)</b>	89.01±1.92	74.48±2.20*	73.97±1.60*	95.66±1.78*	78.45±1.59
<b><math>V_t</math> (ml)</b>	752.3±32.4	799.3±24.0	805.8±15.3*	751.7±23.0	795.9±18.5
<b>RV (ml)</b>	2776.0±149.3	2521.6±94.3*	2504.3±59.8*	2759.8±78.1	2646.8±71.6+

Effects of body posture. Mean values ( $\pm$  SE) of cardiac output ( $Q_c$ ), stroke volume (SV), heart rate (HR), pulmonary tissue volume ( $V_t$ ), and residual volume (RV) in different positions. \* = ( $P < 0.05$ ) difference from pre-tilt standing; + = ( $P < 0.05$ ) from pre-tilt supine.

There were no significant differences in the pre-tilt data between the control sessions, so these sessions were grouped into a single "Pre-tilt" session.  $Q_c$  was elevated (16%) pre-tilt supine compared to pre-tilt standing, and  $Q_c$  during HDT was higher (21%) than pre-tilt standing but not different from pre-tilt supine. Post-tilt,  $Q_c$  dropped immediately in both positions after standing up out of HDT (-28% from pre-tilt standing, -27% from pre-tilt supine), and returned to pre-tilt values by the third

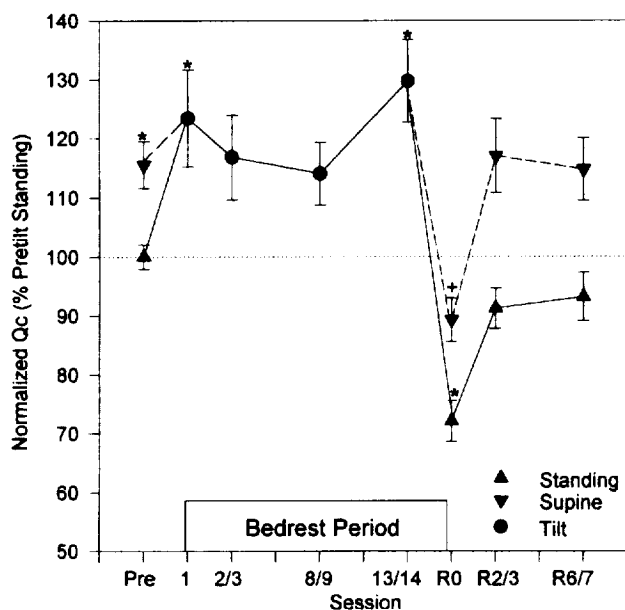


Figure E030-a. Cardiac Output ( $Q_c$ ). Values are means  $\pm$  SE of  $Q_c$  values normalized to pre-tilt standing values. \* = ( $P < 0.05$ ) difference from pre-tilt standing values. += ( $P < 0.05$ ) difference from pre-tilt supine values.

decreased (-5%) during HDT and was just reduced (-4%) pre-tilt supine, compared to pre-tilt standing. There were no temporal patterns seen in either  $V_t$  or RV.

It is known that in the standing to supine or HDT transition, there is a fluid shift from the legs to the thorax (Tomaselli et al., *Aviat Space Environ Med* 58: 3, 1987).  $Q_c$  is also elevated in these two positions compared to standing.  $Q_c$  was markedly reduced immediately (within 2 h) after standing up from HDT. This reduction is thought to be caused by a shift of central blood volume due to gravity from the head and chest regions of the body back to the lower extremities. SV had a similar response to  $Q_c$ , but with larger corresponding changes due to changes in HR in the opposite direction from  $Q_c$ . During HDT, SV started out high and gradually decreased throughout the bed rest period. A similar but nonsignificant trend was seen in  $Q_c$ , as reported previously (Schulz et al., *Acta Physiol Scand* 144:S604: 23, 1992). This decrease in  $Q_c$  and SV has been attributed to the decreasing central blood volume as the subjects adapted to HDT. HR has a trend in the opposite direction to  $Q_c$  and SV, rising during HDT. There was a nonsignificant trend in  $V_t$  to increase in the supine posture along with the increase in  $Q_c$ . A significant increase was seen in HDT. Some increase in  $V_t$  was expected due to an increase in pulmonary blood volume. The small magnitude of the increase seen is indicative of an overall change in extravascular lung water during HDT. Our results are similar to those seen by Schulz et al. (1992), although their results during HDT did not reach the level

day of the recovery period. Compared with pre-tilt standing, SV was elevated (39%) in the supine position pre-tilt, remained elevated (51%) during HDT, and was reduced (-18%) standing post-tilt. SV was also reduced (-12%) post-tilt supine compared with pre-tilt supine. HR was reduced (-15%) pre-tilt supine compared to pre-tilt standing, and was reduced further (-17%) during HDT. Post-tilt, HR values were elevated in both positions compared with pre-tilt values (7% standing, 3% supine). There were temporal changes seen in  $Q_c$ , SV, and HR during the courses of both the HDT period and the recovery period, and all three parameters returned to pre-tilt values in both positions by the end of the recovery period.  $V_t$  was elevated in HDT (14%) compared to pre-tilt standing when the days of HDT were grouped together. No significant changes were seen in  $V_t$  when the days during bed rest were looked at individually. Likewise, RV showed no significant changes in individual days, but taken together, RV de-

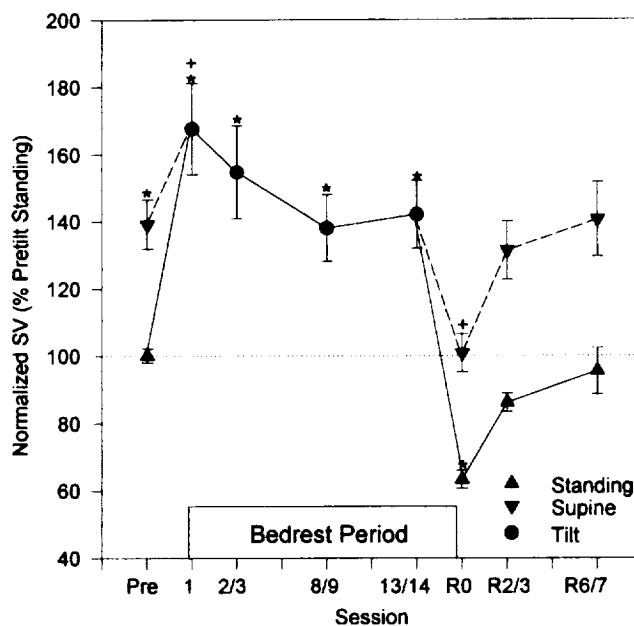


Figure E030-b. Stroke Volume (SV). Values are means  $\pm$  SE of SV values normalized to pre-tilt standing values. \* = ( $P < 0.05$ ) difference from pre-tilt standing values. += ( $P < 0.05$ ) difference from pre-tilt supine values.

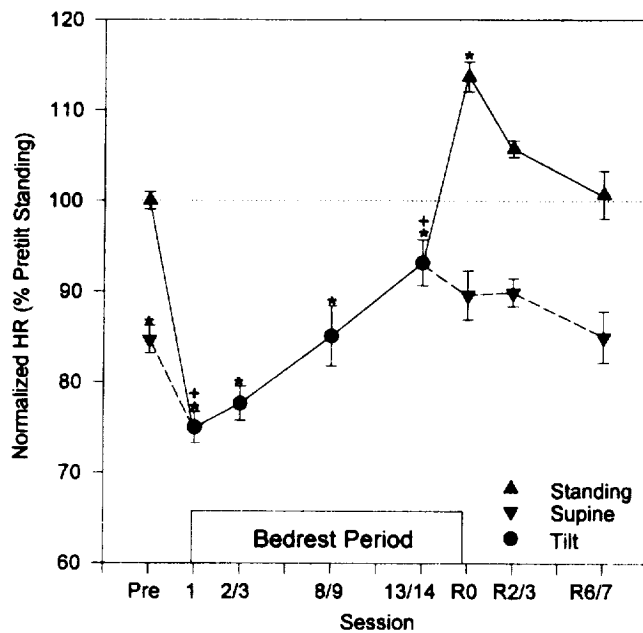


Figure E030-c. Heart Rate (HR). Values are means  $\pm$ SE or HR values normalized to pre-tilt standing values.

\* = ( $P < 0.05$ ) difference from pre-tilt standing values.

+ = ( $P < 0.05$ ) difference from pre-tilt supine values.

yet returned to pre-tilt standing and supine values during 1 wk of recovery. Most parameters responded to the supine and HDT positions as expected, with  $V_t$  and RV showing changes during HDT.

of significance. RV showed little change in HDT and supine. Previous studies had shown no change (Beckett et al., J Appl Phys 61:919, 1986). However, in that study, RV was measured by subtraction of expiratory residual volume from functional residual capacity, which adds noise to the measurement. In this study we began the rebreath maneuver at RV and so directly measured this lung volume in our subjects. We showed a small yet significant decrease in RV in supine control data compared to standing. This reduction was also seen in our ground data collected for the SLS-1 mission (where it was measured by subtraction), but did not reach the level of significance (Elliott et al., J Appl Phys 77: 2005, 1994). The postulated mechanism of the RV reduction in HDT is lung compression due to the weight of the abdominal contents.

Before, during, and after 17 d of  $-6^\circ$  HDT,  $Q_c$ , SV, HR,  $V_t$ , and RV were all affected by HDT,

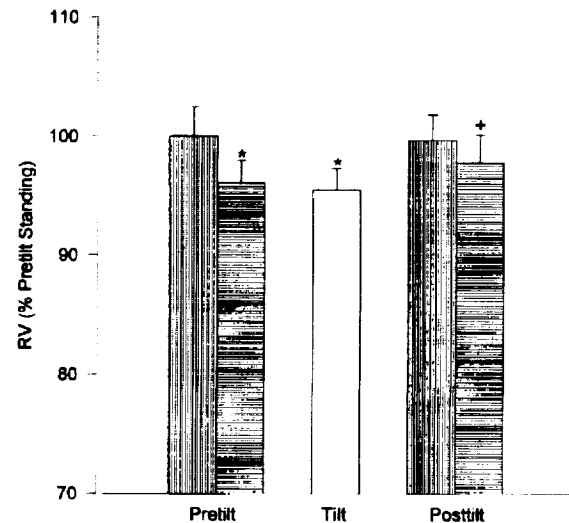
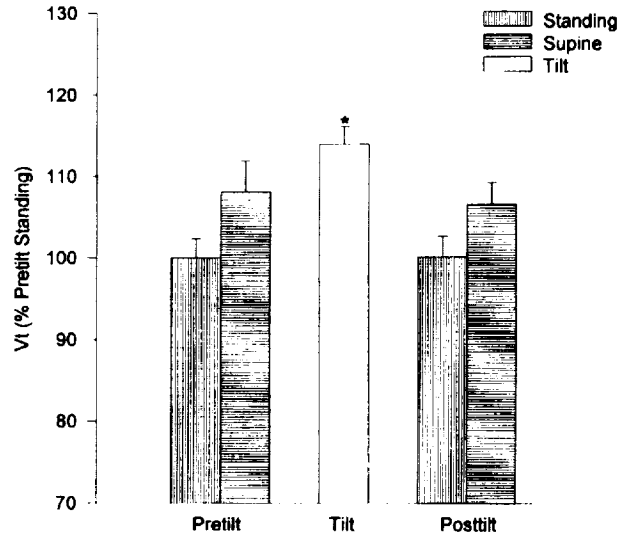


Figure E030-d. Pulmonary Tissue Volume ( $V_t$ ) (left) and Residual Volume (RV) (right). Values are means  $\pm$ SE of  $V_t$  and RV, respectively, normalized to pre-tilt standing values. \* = ( $P < 0.05$ ) from pre-tilt standing values. + = ( $P < 0.05$ ) difference from pre-tilt supine values.

## Measurement of Energy Expenditure During Space Flight: Bed Rest Study

T.P. Stein, Ph.D. and M. Schluter

Although bed rest is generally accepted as a good ground-based model for replicating many of the physiological changes found with space flight, it is possible that strict bed rest with no activity may not be a truly realistic model for space flight for two reasons. Firstly, astronauts are very active whereas with bed rest the activity component is reduced. Secondly, in most bed rest studies the diet is usually controlled during the bed rest phase. The purpose of the present study was to attempt to replicate the LMS space mission on the ground.

In the present study an exercise component similar to that planned for the flight was incorporated into the bed rest phase. The subjects were given enough latitude with their dietary intake that they were essentially on an ad lib diet. The net result will enable us to compare data from a bed rest study, designed to mimic a space life sciences mission where there was a limited amount of physical activity associated with conducting experiments, against an actual space shuttle mission with a similar degree of experiment related activity.

For both the bed rest and space flight experiments, energy expenditure is measured before, during and after space flight/bed rest using the doubly labeled water (DLW,  $^2\text{H}_2^{18}\text{O}$ ) method. Energy balance is calculated from both the difference between intake and expenditure and changes in body composition. Dietary intake and nitrogen balance is measured for the duration (pre, post and in-flight) of the study. Body fat content is determined from both the  $^{18}\text{O}$  space and by DEXA.

### Methods

The protocol is designed to measure energy intake, expenditure, nitrogen balance and body composition before bed rest, during bed rest, and during the recovery phase in blocks of  $6 \pm 1$  d, the same time blocks used in the flight experiment. The time periods are outlined in the upper table. Using such a block scheme increased the sensitivity of the measurements by allowing for duplicate measurements on each subject, an important factor when the number of test subjects is small. Isotopes were given on days L-14, L-9, L+4, L+10, R+5 and R+10, according to the schedule shown at lower right.

During the 47 d of the study, the subjects received all their nutrition from the research center. An attempt was made to provide subjects with a 'controlled' ad libitum diet (see page 4). An accurate record of dietary intake was kept for the entire period. Urines were collected continuously throughout the study.

Each subject ( $n=6$ ) was dosed with  $^2\text{H}_2^{18}\text{O}$  (20 g  $^{18}\text{O}$  and 3 g  $^2\text{H}$ ) for the energy expenditure mea-

<u>Control</u>	<u>Bedrest</u>	<u>Recovery</u>
Block 1 L-14 to L-9	Block 3 L+4 to L+10	Block 5 R+5 to R+10
Block 2 L-14 to L-9	Block 4 L+4 to L+10	Block 6 R+5 to R+10

<u>Elapsed Time</u>	<u>Task</u>
0	Start fast
0.5 h	Collect 1st background saliva
1-2 h	Collect 2nd background saliva
1-2 h	Administer isotopes
6-8 h	Start fast 6-8 h post dosing
6-8 h	0.5 h post start of fast, collect saliva
7-9 h	Start fast
7-9 h	0.5 h post start fast, collect saliva

surements. Two randomly chosen subjects, were not given any isotopes so that their specimens could be used to correct for variations in background water isotopic enrichments that occurred during the study. Saliva was used to sample the body water pool.

## Results and Discussion

This report contains preliminary measurements of energy intake, nitrogen balance and saliva  $^{18}\text{O}$  enrichment. Data in the text, figures and tables are means  $\pm$  SEM. One subject was dropped from the dietary phase of the experiment (intake and N balance) and one subject was dropped from the recovery phase because of illness.

**Energy Intake.** Even though conditions were such as to encourage subjects to maintain a constant dietary intake, subjects made sufficient use of the 'snack basket' to substantially increase their intake for the two ambulatory phases of the study. During the bed rest phase of the study, subjects reduced their intake of snacks. The diet was designed to supply 2500 Kcal of energy and 90 g of protein/d which for an astronaut crew person (avg wt  $\sim$  72 kg) would correspond to  $\sim$  35 Kcal $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ . The mean weight of the subjects in the present study were 10 kg higher.

The combination of providing the subjects with access to a snack basket and not compelling them to eat all the food offered introduced enough latitude for them to adjust their intake needs to the situation. The difference was primarily in the two ambulatory phases where the subjects made more extensive use of the snack basket, although food items were also declined from the set menus. There was a significant decrease in intake for the bed rest phase as shown in the table below and in figure

	Control		Bed Rest		Recovery	
	L-14 d	L-9 d	L+4 d	L+10 d	R+5 d	R+10 d
<b>Total Body Water (TBW) (L)</b>						
Mean (n=6)	45.41	46.34	45.66	46.01	46.48	47.86
SEM	1.25	1.67	1.58	1.32	1.56	1.40
<b>Body Weights on the Days of TBW Determination (kg)</b>						
Mean (n=6)	81.98	81.93	81.35	81.30	81.58	82.13
SEM	3.98	4.23	4.17	4.06	4.02	3.94
<hr/>						
<b>N Balance Summary</b>	n=7		n=7		n=6	
<b>Energy Intake (Kcal/kg/d)</b>						
nominal	30.9		30.9		30.9	
actual	35.9 $\pm$ 2.0		32.8 $\pm$ 1.6		36.3 $\pm$ 2.0	
<b>Protein Intake (mg N/kg/d)</b>						
nominal	169.6		169.6		169.6	
actual	181.8 $\pm$ 9.6		177.9 $\pm$ 8.8		186.5 $\pm$ 10.4	
<b>N Balance (mg N/kg/d)</b>						
actual	21.8 $\pm$ 3.0		1.1 $\pm$ 4.6		27.2 $\pm$ 4.8	

E971-a. It is interesting to note that this intake value (33 Kcal $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ ) is higher than the energy expenditure reported for bed rest without activity (24 Kcal $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ ) (Goran et al., Am J Physiol 266:E510, 1994), but about the same as that found for minimal activity (confined to a bed rest facility), 30 Kcal $\cdot$ kg $^{-1}\cdot$ d $^{-1}$ ) (Gretebeck et al., J Appl Phys 78: 2207, 1995). Since the subjects in this study had several days of intensive exercise one would expect their energy expenditure value to be above that for bed rest alone. How close it is to minimal activity is still to be determined. Independent estimates of the energy costs of the activity will be available from E920.

**Nitrogen Balance.** All values for nitrogen balance, unless otherwise stated, are estimates because they are based on the excretion of nitrogen in the urine and do not include fecal nitrogen losses. As expected, the subjects were in negative nitrogen balance during the bed rest phase of the experiment. They may also have been in marginal negative N balance during the pre-bed rest control period because their activity was somewhat restricted (see table on previous page and figure below). Closer inspection of the data shows that N balance on bed rest days 13-14 was significantly less than on any of the other bed rest days.

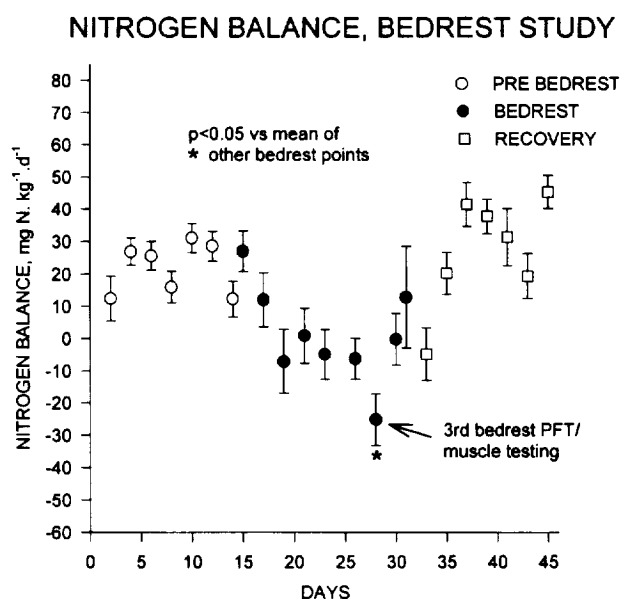


Figure E971-b.

**Total Body Water (TBW) ( $^{18}\text{O}$ ) Data.** At time of writing all of the  $^{18}\text{O}$  measurements have been completed enabling an estimated value for the total body water to be calculated. Remaining to be completed are the urine  $^{18}\text{O}$  measurements for the 6-8 h following isotope dosing to correct the TBW values for isotope lost in the urine prior to equilibrium, as well as the  $^2\text{H}$  measurements.

Body water should track body weight. Thus, if weight decreases as it does during bed rest, the body water should follow. The concordance is poor for the bed rest phase. Overall, for both bed rest TBW determinations, the change in TBW is  $-0.03 \pm 0.21$  L whereas the decrease in body weight is  $-0.63 \pm 0.22$ . The weight loss is significantly different from zero ( $P < 0.05$ ) whereas the TBW is not. The difference in the decrease in body weight and TBW approaches significance ( $P < 0.07$ ). This small but important difference suggests that equilibrium during bed rest is not complete by 8 h post dosing. Most likely water equilibrium in the body proceeds at a slower rate during bed rest because circulation to some parts of the body may be less complete. Assuming the same happens during space flight, the actual energy expenditure rate may be overestimated. To avoid this, we are requesting that NASA collect a saliva sample after 8 h for the LMS mission experiment. Admittedly, the effect is small, but the flight experiment depends on the accuracy of the data. For the bed rest study we have daily saliva samples and it is possible to use the alternate method of extrapolating the daily enrichments back to  $t=0$  h. Daily saliva samples will not be practical on LMS, but the possibility of collecting two more timed salivas is possible.

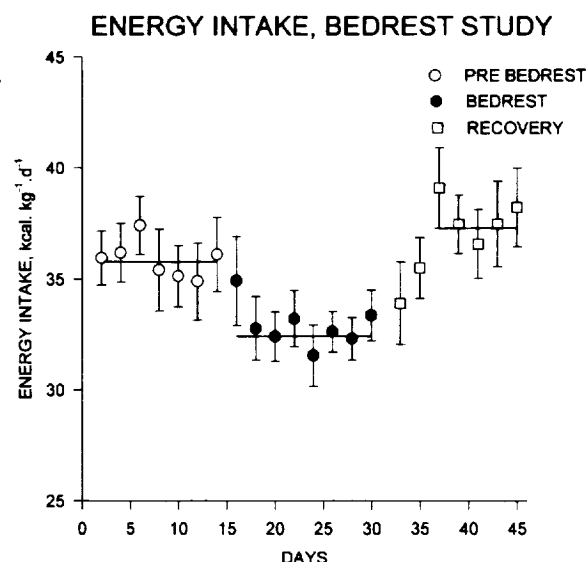


Figure E971-a.

Except for a 1 h escorted walk each day, subjects were confined to the Human Research Facility. Thus just by entering the study subjects reduced their activity levels and this is apparent from their N balance. The interesting point about the bed rest is the apparent outlier point on L+12 and L+13. These 2 d encompassed the last of the pulmonary function tests/exercise protocols (figure E971-b). One possible explanation is that some of the muscles had been weakened by the bed rest to such an extent that the imposition of a strenuous exercise regimen caused some muscle damage.

## Direct Measurement of the Initial Bone Response to Space Flight in Humans

Christopher E. Cann, Ph.D.

This flight investigation consists of three basic objectives. The first hypothesis that calcium is released from the bone very early in space flight, prior to normal osteoclastic resorption, will be tested by defining the time history of calcium release relative to the appearance of bone collagen breakdown products. The second hypothesis that the calcium homeostatic system will adapt to this release of calcium into the blood through normal endocrine regulatory processes will be tested by defining the time course of serum parathyroid hormone (PTH), osteocalcin, and vitamin D metabolites 25(OH)D and 1,25(OH)<sub>2</sub>D and the measurement of calcium absorption. The third hypothesis that the 16-d space flight will stimulate a skeletal remodeling transient with increased bone turnover persisting after flight will be tested by quantifying turnover parameters from 3 mo prior to flight to 2 mo following flight (this hypothesis was not tested in the bed rest study due to its limited duration).



*Technical support provided in the clinical laboratory.*

### Experimental Protocol

The E074 protocol consisted of a number of procedures. A complete metabolic balance study was done from 14 d pre-bed rest (L-14) to 7 d post-bed rest (R+7), consisting of void-by-void urine collections, complete fecal collections (including nonabsorbable fecal marker given during the bed rest period), and continuous monitoring of dietary, fluid and drug intake. From L-10 to R+7, a small amount of a stable calcium isotope (calcium-46) was given with each meal to enrich the normal dietary level of this naturally-occurring isotope, and thus determine how much of the calcium in the blood was coming from the diet, and how much was being released from bone. Blood samples obtained by venipuncture were used to measure ionized calcium, pH, PTH, osteocalcin, and 25(OH)D and 1,25(OH)<sub>2</sub>D.

### Results

The results presented here are those only from the six subjects who had no perturbing influences on the metabolic studies. The preliminary data contained in this report consist of the measures of serum ionized calcium, acid concentration (pH), and urine deoxypyridinoline excretion before, during and after bed rest.

Figure E074-a gives the change in serum ionized calcium measured in morning fasted serum samples obtained on the days indicated on the figure, relative to the control period mean for each subject. The early variability in Ca<sup>2+</sup> is probably due to effects of hemoccentration, but there is a very slight elevation in Ca<sup>2+</sup> which persists during bed rest and returns to normal during the recovery period.

Figure E074-b shows the change in the measured serum hydrogen ion concentration, as determined from the measured pH in the samples. The values were determined using a selective ion electrode (as were the Ca<sup>2+</sup> values) on serum obtained using the flight blood processing protocol. This protocol involves first centrifuging the blood samples in serum separator tubes followed by immediate freezing, with determination of the pH and Ca<sup>2+</sup> on the serum obtained after rapid thawing and anaerobic



removal from the tubes used to draw the blood samples. Thus, this protocol does not allow for the exposure of the specimens to air. The freeze-thaw cycle, however, may cause small amounts of free CO<sub>2</sub> and other gases in the serum to be driven out of the serum into the small remaining free space in the tube, and although the gases cannot escape they may not completely redissolve in the serum before its removal. If this is the case, the pH measured using this protocol would be expected to be slightly higher than the normal 7.4; in fact, this was the case, with a mean control pH of 7.58. The figure shows that there is a significant increase in serum [H<sup>+</sup>] during bed rest which returns to normal following reambulation. This increase corresponds to about a 0.05% decrease in pH, but is expressed here in absolute terms rather than using the logarithmic pH scale.

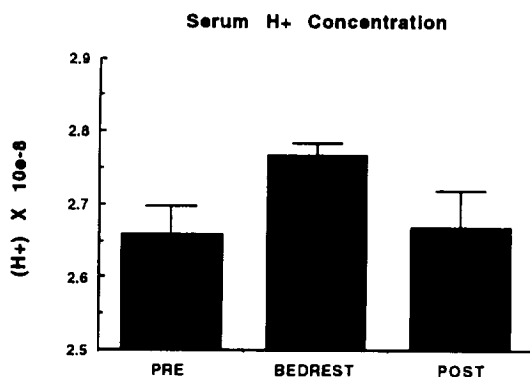


Figure E074-b.

### Discussion

The preliminary results of this bed rest study given in this report are consistent with a rapid slight increase in serum calcium which can lead to a series of adaptive endocrine responses. The role of the increased serum H<sup>+</sup> is not known, whether it is causative of the increased free Ca<sup>2+</sup> or a response to other effects such as hemoconcentration. Its magnitude is significantly lower than that observed in samples obtained and processed in space, suggesting that the results observed from SLS-1 and SLS-2 may be unique to space flight, either in the pH of peripheral venous blood or in the behavior of the blood as it is being processed. However, strict adherence to the blood processing protocol used in space for this bed rest study did not cause the H<sup>+</sup> increases noted in the SLS-1/2 samples, suggesting a physiologic cause.

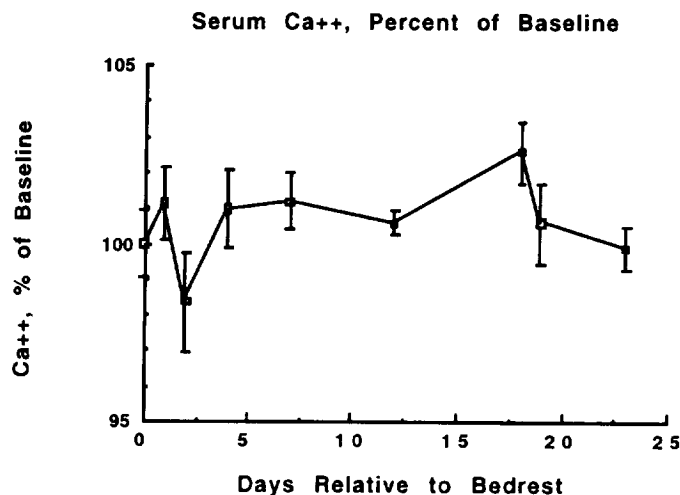


Figure E074-a.

Figure E074-c gives the average urine output of deoxypyridinoline crosslink collagen degradation products in the first morning urine, normalized to urine creatinine. As expected, there is a clear increase of DPD with bed rest. The continued increase in the recovery period is also expected, presumably caused by the increased bone resorption persisting during the 2-3 wk post-stimulus period during which the new osteoclasts continue to resorb bone.

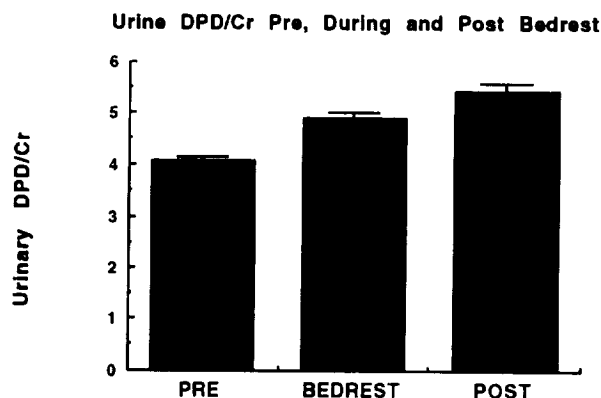


Figure E074-c.

The urine DPD/Cr results seen in this bed rest study are of a similar time response, though lower in magnitude, than similar results obtained from SLS-2. The results from space flight show about 40-50% increase in DPD, while the bed rest increase was only about 20%. In space flight, the increase in DPD was delayed until about 5 d into flight, with a similar effect seen in this bed rest study (data not shown). This indicates that the basic process of increased activation of bone turnover in response to unloading pertains in both situations, although the bed rest response may be blunted because the body is still under significant gravitational influence even though the gravitational vectors may be altered. The SLS-2 urine DPD remained elevated until at least 2 wk post-flight; the metabolic portion of this bed rest study terminated after 1 wk of reambulation, but urine DPD was still elevated, consistent with the space flight results.

The preliminary results obtained from this 17-d bed rest study indicate that the parameters chosen to be measured during the LMS mission show changes even during a ground-based simulation, with the ground-based results showing a comparatively smaller response than what would be expected in space flight. There are still some differences noted between bed rest and previous space flight results, clearly of a quantitative nature and perhaps of a qualitative nature as well. However, using the anticipated flight timeline for this bed rest study did not uncover any interactions with other experiments which would be expected to significantly influence our results and therefore compromise the outcome of our experiment.

# Performance Studies

## Overview

**F**undamental to the performance of physical work is the integrity of the central nervous system (CNS). Aside from the neural feedback of muscular activity to the CNS, bed rest or microgravity impose environmental conditions that influence the capacity of the CNS to perform at peak efficiency. In the space environment the absence of day and night terrestrial cues and differences in the physical activities of crews impose a situation in which the Earth-based biological clock may not function for optimum performance. In fact, previous studies have shown that exposure to altered gravitational environments can have profound effects on both the expected level of regulated rhythms and also on circadian rhythms themselves. Disruptions in circadian rhythms not only adversely affect an individual's ability to respond to environmental challenges, but also decrease performance and contribute to psychological disorders. Conditions such as jet-lag, performance problems and some sleep and mental disorders are frequently associated with disruptions in the habitual circadian rhythms. Interrupted sleep occurs commonly in astronauts from noise, discomfort or external stress. It is difficult to know to what extent the fatigue resulting from inadequate sleep contributes to the muscle weakness. Likewise, the relationship between alteration of circadian rhythms and level of performance is unclear. Objective measures of performance requiring verbal, social, and cognitive skills as well as subjective estimates are required to properly evaluate the role of circadian rhythms in muscle weakening.

Two investigations were carried out to assess the impact of bed rest on cognitive performance (E963 Dr. Schiflett and E948 Dr. Monk) and on eye movements (E073 Dr. Reschke). Performance tests (E948) that measured the speed and accuracy of visual search and reasoning abilities were carried out before each meal during a 72-h time period, during the control, bed rest, and recovery periods, when the circadian rhythms study was being conducted. Dr. Schiflett's computer based investigation provided information on specific brain reasoning processes that could be affected by bed rest and/or microgravity, i.e., information encoding, retrieval from long-term memory, etc. Dr. Schiflett's battery of tests was not linked with the studies of circadian rhythms but was carried out at regular intervals within each of the test periods in sequence with other scheduled muscle and metabolism tests.

Already carried out in space on Shuttle mission IML-1, Dr. Reschke's investigation of eye movements in microgravity had not been performed during a bed rest study. Physiologic eye movements were monitored by electrodes placed above the right eye, over both right and left temples, below the right eye, and behind the left ear. The optokinetic goggles present a pattern and the computer program records the eye movement data while the subject is seated, supine, and lying on the right side before and after bed rest, and while supine and lying on the right during bed rest. The protocol consisted of a series of observations of a moving pattern triggered by the operator. The subject was tasked to gaze straight ahead at the moving pattern. The eye movements (nystagmus) generated by tracking the target were recorded during a series of pattern changes that were interrupted by brief periods of darkness and stationary displays.

## Investigators

### PERFORMANCE

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**Pittsburgh, PA**  
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**R.E. Schlegel, Ph.D.**  
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**R.L. Shehab, Ph.D.**

**E073 • M.F. Reschke, Ph.D.**  
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**W.P. Huebner, Ph.D.**

## Preliminary Report of SACS LMS Bedrest Study

Timothy H. Monk, Ph.D.

*Design and Method*

Three 72-h measurement blocks were taken, one during the ambulatory pre-bed rest baseline (L-6 to L-4), one during early bed rest (L+4 to L+6), and one at late bed rest (L+14 to L+16). For rhythmic measures (body temperature), a 72-h plot of the variable against time (three circadian cycles) was made. Core body temperature was sampled every 6 min for 72 h at each measurement block with a rectal thermistor. Each subject's rhythms were then quantified using sinusoid-fitting techniques which yielded estimates of amplitude (size of rhythm) and acrophase (timing of the fitted rhythm peak) at each of the three measurement blocks (pre-bed rest or control, early bed rest, late bed rest).

For sleep measures (both objective diary-based) the measure was averaged over nights two and three of the three-night set within each measurement block, taking the first night as an adaptation night. Objective sleep measures were made using standard sleep scoring conventions applied to each individual minute of sleep. Characteristics of sleep were captured by a questionnaire.

In all cases individual endpoints were calculated for each subject, and the data averaged over the eight subjects to yield means and standard errors at each measurement block. Equipment failure caused the loss of temperature data from one subject and of two separate subject-nights of sleep. Statistical significance was tested by one-way repeated measures ANOVA.

Sleep recordings were obtained with a Medilog ambulatory sleep recording device worn as a belt pack. Connected to this device were eight electrode leads. Four measured evoked potentials (EEG) (C3, C4, with A1 and A2 as reference), two lateral eye movements using electrooculography (EOG) at the right and left outer canthi, and two electromyograph leads (EMG) from the chin.

*Body Temperature Results*

Rhythms are plotted on the next page (mean  $\pm$  SE) (figure E948-a). There appeared to be a statistically reliable decrease in rhythm amplitude, and phase delay in rhythm timing as bed rest progressed (figures E948-b and c). Temperature rhythms became progressively flatter (by about 25%) and later peaking (by about 100 min). This indicates that the circadian system (biological clock) was disrupted during head-down tilt. Interestingly, the phase delays observed in this study were very similar to those observed by Dr. Alex Gundel and colleagues in a study of a German astronaut aboard Space Station Mir.

*Objective Sleep Recording Results*

There was a statistically reliable change over the three measurement blocks in the time spent actually asleep at night. This appeared as a decrement

in the early bed rest measurement block and was particularly apparent when the percent night awake (a measure of insomnia) was calculated (figures E948-d and e). As one would predict from the late phasing temperature rhythms, this insomnia occurred in the beginning part of the night, taking the form of an increase in sleep latency (the time taken from first trying to fall asleep to actually entering sleep). There was no reliable difference between the three measurement blocks in the time spent awake after sleep onset, or in the amount of delta (deep) sleep. There was, however, a suggestion of

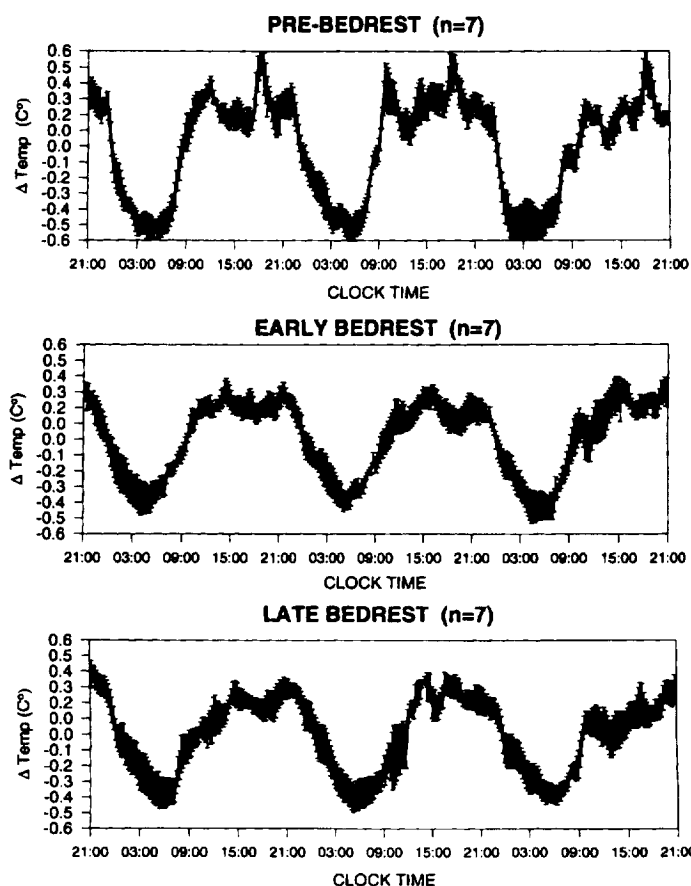


Figure E948-a.

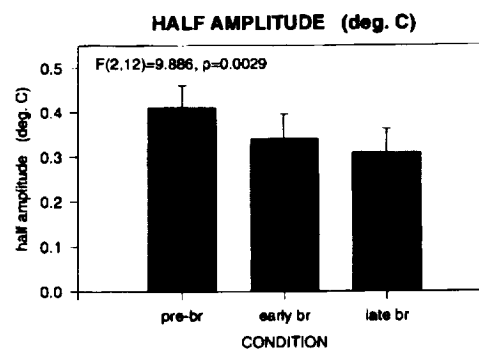


Figure E948-b.

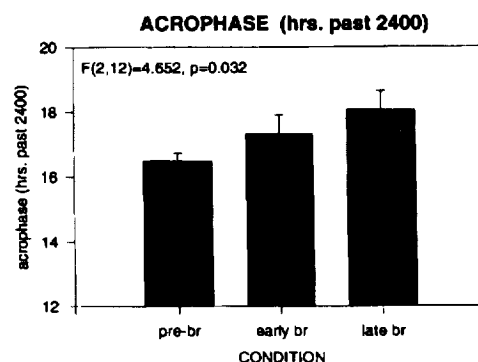


Figure E948-c.

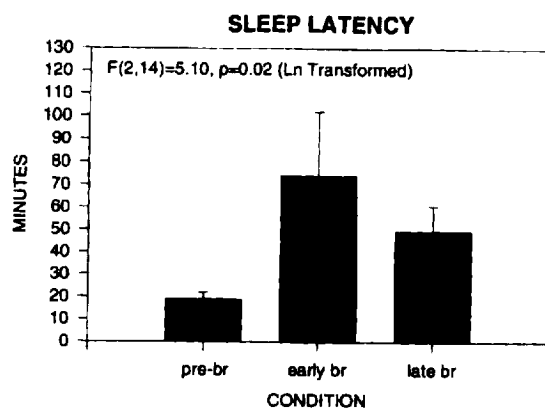


Figure E948-d.

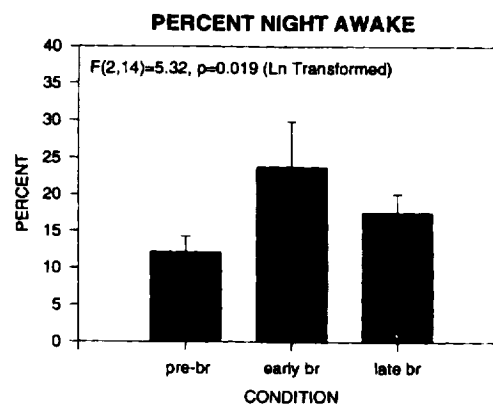


Figure E948-e.

an increase in the amount of stage REM (dreaming) sleep, and a decrease in REM latency (a measure of how soon after sleep onset the first REM of the night appears) (figures E948-f and g).

### Sleep Diary Results

The sleep diary results followed the objective measures, with a statistically reliable lengthening in number of minutes to fall asleep, and self-reported sleep quality, which was particularly evident in the early bed rest measurement block (figures E948-h and i).

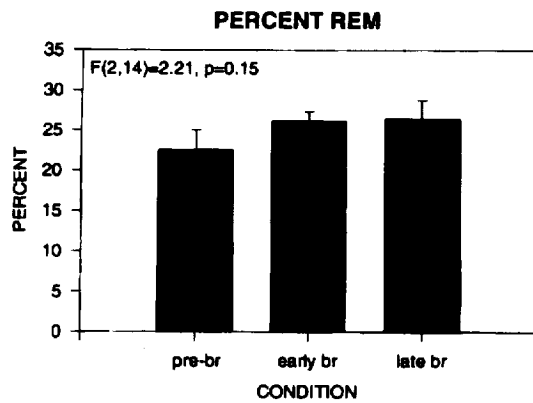


Figure E948-f.

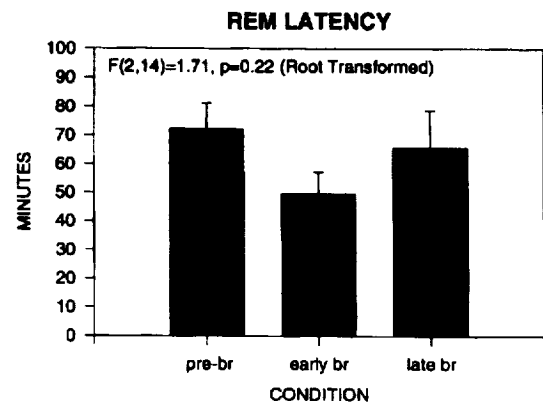


Figure E948-g.

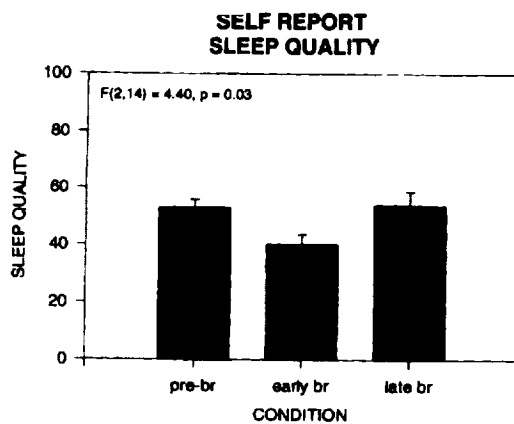


Figure E948-h.

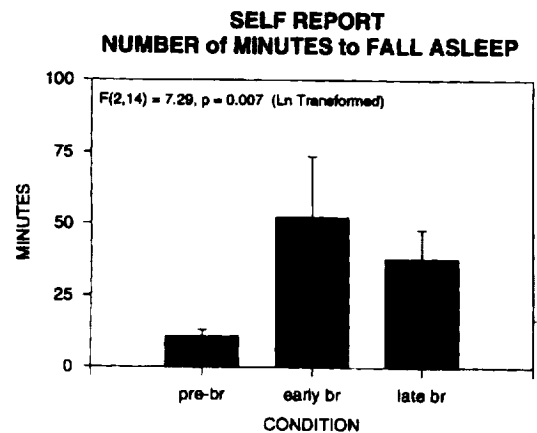


Figure E948-i.

## Conclusions

The study was successful in demonstrating circadian abnormalities related to head-down tilt bed rest. These abnormalities took the form of a progressive flattening and phase delay in the circadian body temperature rhythm, and were associated with both subjective and objective insomnia during the early part of the night, particularly in early bed rest. There was also suggestive evidence for an increase in REM (dreaming) sleep, and an earlier occurrence of REM sleep in the night when bed rest was compared to control.

## Microgravity Effects on Standardized Cognitive Performance Measures

Robert E. Schlegel, Ph.D., Randa L. Shehab, Ph.D., Samuel G. Schiflett, Ph.D., and Douglas R. Eddy, Ph.D.

The NASA Performance Assessment Workstation (PAWS) presents a 20-min battery of six cognitive assessment tests and two subjective scales. The performance tests assess directed and divided attention, spatial, mathematical, and memory skills, and tracking ability. The subjective scales assess overall fatigue and mood state. The PAWS has been used to evaluate flight crew performance on the Second International Microgravity Laboratory space shuttle mission (IML-2) and is scheduled for use on Life and Microgravity Spacelab.

For this bed rest study, PAWS was implemented on an IBM Thinkpad 755C and consisted of the following tasks in order.

Monk Mood Scale - requires a response on a linear graphic scale with endpoint descriptors of "very little" and "very much" for each of the eight mood categories: Alert, Sad, Tense, Effort, Happy, Weary, Calm, and Sleepy.

Critical Tracking - involves tracking an unstable object on the display using a 2-in trackball (MSI Model 622) for 2 min.

Spatial Matrix - involves indicating whether a matrix of squares is the same as one previously presented. The test lasts 1.5 min.

Sternberg Memory Search - involves indicating whether a letter is the same as one of those in a previously memorized set. The test lasts 2 min.

Continuous Recognition Memory - involves pressing a key to indicate whether a number is the same as one previously memorized. The test lasts 2 min.

Switching Task - involves responding to 1 of 2 tasks presented simultaneously on each screen display. In the Manikin task, the subject presses a key to indicate which hand of a manikin holds a matching symbol. In the Mathematical Processing task, the subject presses a key to indicate whether a sum of three numbers is greater or less than 5. The test lasts 4 min.

*During bed rest, subjects performed the PAWS protocol while lying on a gurney with their head resting on a padded "doughnut".*



Dual Task - involves performing the Sternberg Memory Search while Tracking and lasts 3 min.

Fatigue Scale - involves pressing a numbered key to indicate which statement best matches the subject's fatigue state and takes less than 15 s.

## PAWS Test Protocol

Following orientation and eight training sessions (two sessions per day), subjects completed 16 additional practice sessions (two sessions per day). The last eight practice sessions were performed with subjects lying face-down on a gurney to minimize the potentially confounding effects of different subject positioning during bed rest testing. During bed rest and recovery, subjects performed one session every other day. Testing was conducted bedside, with the subject either seated at a small table or on a gurney with the test apparatus supported above the floor at an appropriate height. When not in conflict with the scheduling constraints of the other experiments, testing was conducted within 4 h of waking. Additional constraints included minimal food intake prior to testing and no exercise at least 30 min before testing. Attempts were made to minimize distractions and noise in order to provide a quiet test environment. In most cases, these attempts were successful.

Switching - Manikin Tas

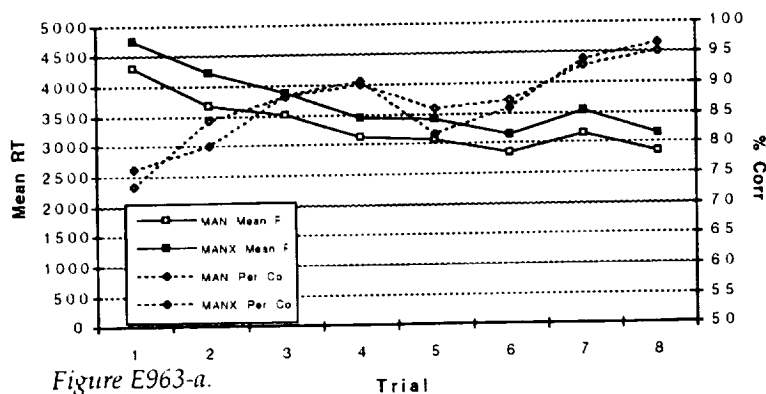


Figure E963-a.

Dual - Tracking

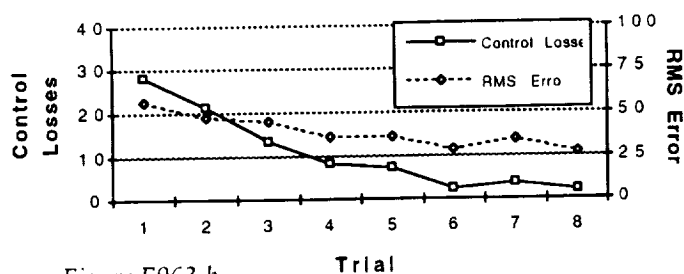


Figure E963-b.

Matrix

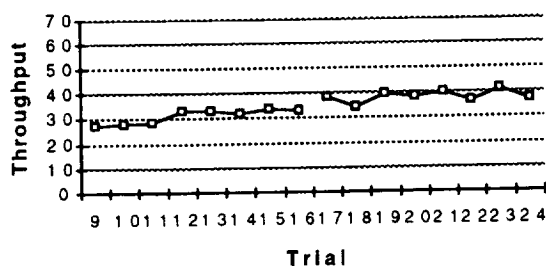


Figure E963-c.

## Results

**Training.** Figures E963-a and b present the typical pattern of performance improvement during training. For discrete response tasks, response time becomes faster (decreases) and percent correct increases. Tracking improvement is reflected in fewer control losses and

**Control Period.** Figures E963-c and d illustrate the modest but continued improvement in performance as subjects become more proficient while establishing individual baselines. The throughput measure for the Matrix and other tasks represents the number of correct responses processed per minute. Note the continued improvement in spite of the transition from the seated test

Dual - Tracking

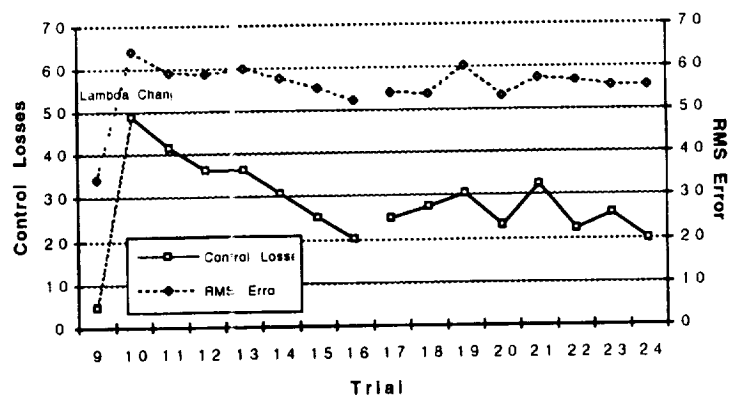


Figure E963-d.



position to the horizontal gurney position. For Dual-Tracking, the worsening of performance from Trial 9 to Trial 10 is due to a significant increase in the difficulty of the task. Note how the steady improvement from Trial 10 through Trial 16 is halted by the change to the horizontal testing position. A slight performance loss during the transition was also observed for the Critical Tracking task. Also, on the Switching task elements, performance improvement ceased or performance actually declined.

**Bed Rest.** Figures E963-e through h summarize average performance for all three test periods. T represents an average of the last two training trials, P1 through P16 illustrate four specific trials during the control period (including the transition from seated to gurney testing - Trials P8 and P9), BR3 through BR17 present results from specific bed rest days, and R0 through R+6 represent the recovery period. In general, there was no apparent cumulative effect of bed rest. Following a short period of performance stabilization, the commonly observed trend of slight but steady performance improvement occurred. For most tasks, this performance improvement was enhanced during recovery. With respect to Dual-Tracking, the performance improvement (control losses) is somewhat erratic and does not stabilize until recovery (figure E963-f). The same pattern may be observed for throughput in Dual - Memory Search (figure E963-g). Although no statistically significant differences in performance were observed when comparing bed rest with the control period, additional analyses must be conducted to determine if the rate of performance improvement was slowed during the bed rest period. On average, Fatigue scores showed little change across all periods, with the exception of a dramatically lower fatigue level on day R+4 as the subjects readapted to a more "normal" living pattern.

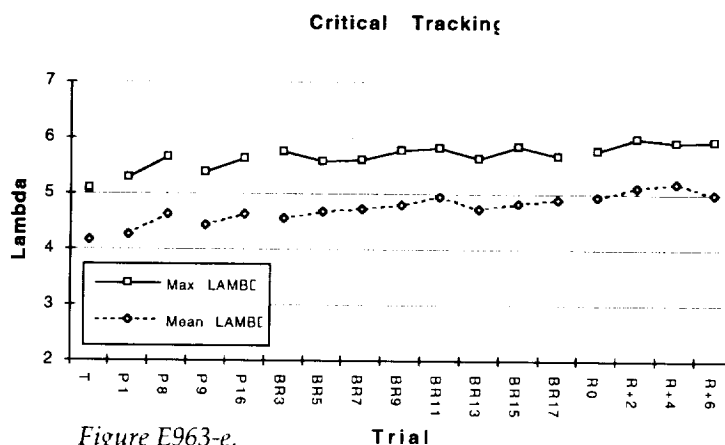


Figure E963-e.

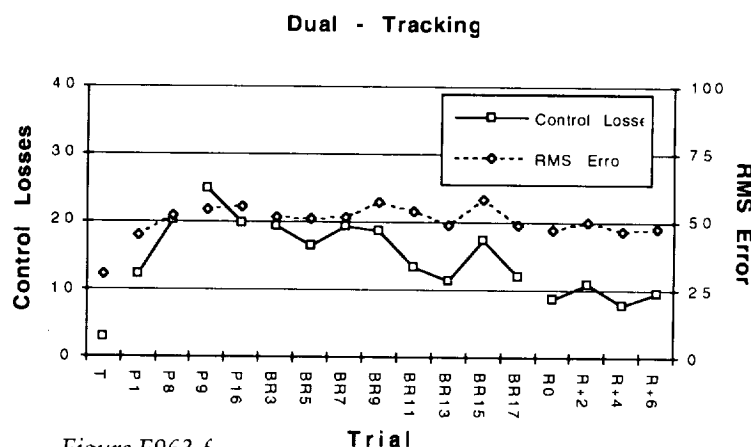


Figure E963-f.

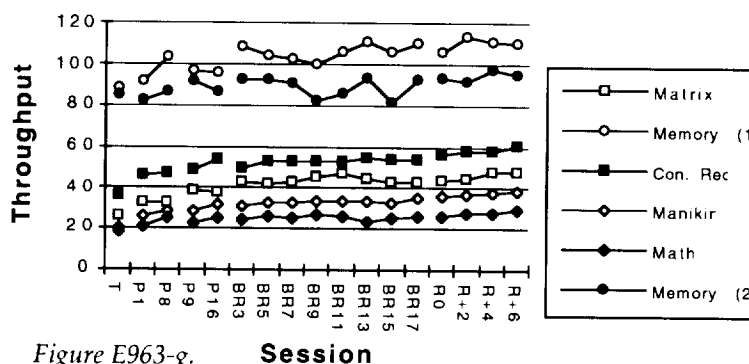


Figure E963-g.

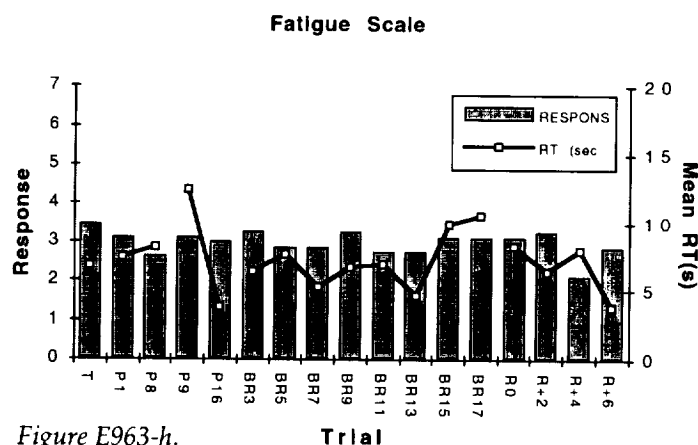


Figure E963-h.

**Conclusions.** Overall, the effect of prolonged bed rest on cognitive performance was minimal. In many cases, the effect was manifested as a decrease in the already slight rate of performance improvement. The effect on the motor control tasks was more substantial, but much of this effect could be attributed to differences in testing positions. It is important to remember that these data presentations represent group means. Assessment of the individual effects of bed rest on each subject remains to be completed. For the PAWS flight experiment, this bed rest study provided data on an important ground-based control group. Any performance changes observed on orbit can be reasonably assumed to be the result of the microgravity space environment.

## Canal and Otolith Studies

Millard F. Reschke, Ph.D., William P. Huebner, Ph.D., Linda Michaud, and Carol Verret

*This study used electro-oculographic (EOG) recording (right) to measure eye movements with the body placed in three positions; seated, standing, or supine to quantify any spontaneous and/or positional nystagmus which might be present before, during and after bed rest.*



1.

*Specially constructed opto-kinetic stimulus (OKS) goggles (below) consisted of a visual display of alternating black and white stripes embedded in a goggle housing which attaches to the head with a webbed strap. When in motion, the pattern moved continuously in the subject's field of view.*



4.

*A specially constructed plywood frame that has a floor or foot rest directed to an angle  $30^\circ$  from the normal horizontal floor and padded interior side surfaces was used for the standing position (left). Six degree head down tilt was used for the supine position.*



2.

Prior to and following bed rest, this test was conducted with the subject in the following ordered positions: 1) seated with head upright; 2) seated with head tilted  $30^\circ$  to the right; 3) seated with head tilted  $30^\circ$  to the left; 4) standing in the tilted frame with the long axis of the body tilted to the right; and, 5) standing in the tilted frame with the long axis of the body tilted to the left. During bed rest, the test was conducted with the subject lying on the right side.



3.

*The orientation of the pattern with respect to the subject could be vertical, horizontal or at an angle of  $45^\circ$  (left). The pattern motion was computer controlled by custom software on a Macintosh computer that also recorded eye movement data. A straight-back chair was used for the seated position.*

# Value of Bed Rest Pilot Study

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## Investigator Questionnaire

While the value of the bed rest study cannot be firmly determined until after the mission, the opinions of the participating scientists on the effect of the pilot study on their preparation for the flight experiment was assessed by a questionnaire. The responses of each experimental group to the eight questions posed are summarized.

## *Equipment*

The need to use different equipment was considered a major problem to a pilot study carried out in Northern California at a time when equipment was in the process of being set up and transferred to Florida for the flight.

All four muscle experiments requiring the compact Torque Velocity Dynamometer (TVD) for muscle testing had to substitute another device, either the Cybex (E920) or the LIDO (E036, E407, E401) each of which used a different data acquisition system, updated and revised for the LMS experiment. Either identical (E409) or similar (E029) equipment was used by the other two muscle experiments.

Two of the three metabolic experiments used different equipment and reagents. Pulmonary function (E030) was measured with laboratory hardware that performed similar functions and, in the absence of automation, each test was performed manually. A different stable isotope was given for the calcium metabolism experiment (E074) and a typical clinical laboratory used for processing blood samples instead of the equipment used in the shuttle.

Performance testing was accomplished with equipment identical to that to be used in flight (E963, E073), except for a cap, custom fitted for each crew member, used for collecting electroencephalography data (E073).

While only 4 of 12 experiments used the same equipment in the pilot study as in the flight experiment, the same form and type of data were acquired that could be used for comparison with flight data except for one experiment where the limitations of the LIDO resulted in a different type of data (E407) (see Integrated Muscle Studies for details).

## *Testing Sequence and Protocol*

The testing sequences and protocols were modified as little as possible to accommodate twice the number of subjects for testing (8 vs. 4), the main reason for the adjusted schedules. There were also the considerations that scientists, not subjects, carried out the experiments and equipment was limited. To avoid some schedule conflicts, subjects were admitted in two groups, with 2 d intervening. Nevertheless, there were substantial differences in the schedules of 8 of the 12 experiments.

The testing sequences of the muscle experiments were coordinated through the development of an Integrated Muscle Testing protocol (see appropriate section). This protocol successfully compressed the testing sessions for two subjects to 1 d with the same sequence carried out in two more subjects the following day. The time sequence for one individual allowed ample time for the eight subjects to be tested on two separate occasions during the control period, three times during the bedrest and three times during the recovery period. A similar test sequence with two subjects on one day and two subjects the next day for the pulmonary function tests with exercise was carried out. Once this 2/2 time sequence was established, it set the schedule for venipunctures connected to the muscle experi-

ment testing growth hormone stimulation (E036) and was also used by the other experiment (E074) requiring blood to minimize the general discomfort of needle sticks. This coordinated effort reduced the number of days when muscular activity including fatigue tests were required, and improved the opportunity to quantify the expected physiologic effects of bed rest, i.e. muscle atrophy, loss of fluid, lean body mass etc. It also enabled a few experiments to occupy an entire 24-h period for measurements without the potential interference of 'activity' data on 'rest' data in experiments involving sleep, circadian rhythms, and performance (neuroscience).

The lack of similarity in bed rest compared to flight schedules for a number of experiments was the result of establishing the muscle experiments as the highest priority with other experimental testing schedules adjusted to the schedule of the integrated muscle testing protocol (E948).

Some differences between flight and bed rest schedule were related to the problem in acquiring physiologic data (i.e. blood for E074) during the orientation period, a poorly controlled period for subjects who came to the unit for training. One difference took advantage of the 17th bed rest day to acquire data before subjects were upright to observe the full effect of bed rest. Two experiments had fewer measures before and after the 45 d experimental period than are planned for the flight experiment, largely because of the short amount of time available for gathering subjects and their consent (E029 and E074).

### ***Value of Study to Scientists Science Program***

The value of the pilot study to each investigators science program was rated as high in 8, moderate in 3 and low in 1. Reasons cited for high value were that it identified problem areas for the flight experiment, solved a number of issues relating to multiple experiments, stimulated the science program, provided data that could be used for comparison with flight data and as well as a more meaningful statistical analysis with a larger number of subjects than in the flight experiment. There were some reservations about the value of the study because the bed rest model was not considered optimal and the experimental design did not include a group of concurrent control subjects (E074). Bed rest was not considered a provocative stimulus to one experimental system (E073).

### ***Value of Study to Projected Flight Project***

The value of the pilot study to the flight experiment was considered high in nine experiments because of the opportunity to refine experiments, smooth out procedures, identify problem areas, or change minor aspects of the experiment. Opinion was divided regarding the value of the opportunity to get to know the other investigators in the mission.

### ***Experiment Impact on Another Scientist's Experiment***

The impact of one experiment on another was considered positive by four (E029, E963, E971, E409) because of data sharing for related responses or potential prediction of responses. Others (E920 and E030) reserved an opinion on an interactive experimental effect pending data analysis. No firm negative impacts were expressed but a possible conflict relating to timing of a measurement in two experiments was identified (E029 and E409).

### ***Effect of Schedule on Interactive Effects of Experiments***

Interactive effects of experiments were not necessarily prevented by the bed rest study schedule which, for the most part was considered 'satisfactory' or 'acceptable but not optimal'. The flight timeline was not followed precisely. Three experimenters could not determine whether the schedule prevented interactive effects and two considered that it did.

## *Changes in Flight Protocol Based on Bedrest Study Experience*

Half of the scientists will not make changes in the flight experiment based on experience with the bed rest study (6 of 12). Four experiments had minor revisions made based on the pilot study (E036, E030, E971, and E948) and two reserved comment pending completion of data analysis.

## *Evaluation of Overall Performance of the Study (Facilities, Personnel, Volunteers, Assistance Provided)*

The overall performance of the study at ARC was considered high with respect to facilities and personnel. The problem of trying to acquire sensitive electronic data in a room that is not isolated from the building electrically was mentioned as a difficulty by two scientists.

## *Publications From This Study*

All investigators thought that at least one publication would be generated by the data collected during their study.

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## **Summary**

The pilot study demonstrated very well the feasibility of carrying out a number of planned experiments collectively without much difficulty. The integrated schedule for four muscle experiments, developed for this study, was critical to this achievement. Carefully planned daily schedules for two groups of four which allowed for experiments to be repeated at the same time of day for each subject while maintaining a regular meal schedule contributed to the success of all the experiments.

A major concern at the beginning of the study was partially resolved. Both the intensity and frequency of exercise from the muscle testing protocols may well have been enough to act as a countermeasure for the problem under investigation (muscle atrophy and weakness). Calf muscle strength and fatigue characteristics in two experiments showed little effect of bed rest (E920 and E407). Some effect of the model was observed in E401, an experiment that did not rely on voluntary contractions. The thigh muscle (quadriceps) tested before and after bed rest showed an 8% decrease in the strength of maximum voluntary contraction. E036 also observed a bed rest effect in the response of circulating tibial growth factor stimulated by exercise. While some of these responses were considered less than expected for the protocols, the results form a strong database of the combined set of experiments planned for the mission for realistic comparisons.

Additionally, E029 acquired data indicating that the exercises that were a part of the muscle testing experiments, did not prevent loss of lean body mass. The small, but significant, decrease in lean body mass agreed with the independent preliminary measures of nitrogen balance from E971 which were negative during bed rest. As well, E074 found an increase in the urinary excretion of deoxypridinoline, a product of bone collagen reflective of increased resorption. This is evidence that the exercise protocols did not entirely counteract the unloading effect of the bed rest model. The depression of body temperature from E948 also showed a bed rest effect not compromised by the active muscle testing protocols.

One identified problem in the design of the metabolic studies for the flight experiment was the collection of urine specimens by spontaneous voids instead of time, i.e., 24 h. While most of the specimens were actually 23-25 h and could be used for estimates of daily excretion, the mean values in the tables

in this report reflect 70-72% of daily specimens from four subjects and more than 89% of specimens in the other four. Voids at the same time each morning to begin and end a 24-h collection of spontaneous voids would simplify data analysis of compounds in urine.

Preliminary results from one neuroscience experiments carried out in 72-h blocks on non-muscle testing days (E948) revealed both subjective and objective evidence of insomnia during the early bed rest. Another experiment that checked cognitive performance with high enough frequency to compare the performances (E963) on days with and without intense muscle testing did not reveal a negative impact of intense exercise on the cerebration of the subjects.

We await with interest the results of the space flight experiments for confirmation of the head-down tilt bed rest as a model for space flight.

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